University of Alberta

NUTRITION-REPRODUCTION INTERACTIONS IN CYCLIC GILTS

by

Fernanda Radicchi Campos Lobato de Almeida ©

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The undersigned certified that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *NUTRITION-REPRODUCTION INTERACTIONS IN CYCLIC GILTS* submitted by *FERNANDA RADICCHI CAMPOS LOBATO DE ALMEIDA* in partial fulfillment of the requirements for the degree of *DOCTOR OF PHILOSOPHY* in *ANIMAL SCIENCE.*

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ABSTRACT

The mechanisms by which nutrition and metabolic state affect fertility in cyclic gilts were studied. Experiment 1 tested the impact of different patterns of feed restriction between d 1 and 15 of the estrous cycle on subsequent fertility. HH gilts were fed 2.8 X maintenance throughout the cycle, in contrast to restriction (2.1 X maintenance) in the first (RH) or the second (HR) week of the cycle. Embryonic survival was reduced ($P < .05$) in HR gilts, without affecting ovulation rate. HR gilts also had lower ($P < .05$) plasma progesterone concentrations at 48 and 72 h after onset of estrus compared to HH and RH gilts.

In Experiment 2, RH and HR gilts were bred by AI and fertilization was allowed to occur *in vivo*; early fertilized oocytes were then recovered for culture *in vitro*, to assess the effect of the patterns of feed restriction on embryonic development. There was no effect of treatment on embryo developmental competence, however fertilization rate was lower ($P < .06$) in HR compared to RH gilts. Ovulation rate was again not different in the RH and HR treatments, but no differences in plasma progesterone were observed.

In Experiment 3, the responses to insulin treatment during the period of feed restriction in the late luteal phase that had deleterious effects on subsequent fertility were investigated. Feed restriction did not affect plasma insulin, IGF-I, leptin, total and freeT3, nor plasma LH, FSH, estradiol and progesterone on days 15 and 16 of the treatment cycle. In the subsequent peri-estrus period, plasma concentrations of

LH, FSH, estradiol and progesterone were higher ($P < .05$) in RH and HR+I compared to HR gilts. There was again no treatment effect on embryo development.

These results demonstrate that feed restriction during the later part of the estrous cycle had lasting effects on ovarian function and endocrine status in the peri-estrus period, which may mediate differences in embryonic survival to day 28, apparently irrespective of effects of treatment on the developmental competence of fertilized oocytes.

Aos meus pais, Egladson e Lourdes, meu
marido, Leonardo, e meus filhos Guilherme e
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grandes incentivadores da minha carreira,
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CHAPTER 1

INTRODUCTION

Reproduction in mammals is a complicated process that must occur in harmony with existing dietary, physical and social conditions. Among the environmental factors that can influence reproductive activity, including photoperiod, temperature and social cues, food availability can be considered as playing the most important role (Bronson, 1985; Bronson, 1989). In the wild, all adult mammals must forage for their food, they must assimilate energy from that food, and then they must partition the use of that energy among interacting and often competing demands. The demands that must be satisfied first are cellular maintenance, thermoregulation and locomotor costs of obtaining food. Once these primary demands have been satisfied, the remaining energy can be allocated to growth or to the physiological and behavioral demands of reproduction, or it can be stored in the form of fat (Bronson, 1985). Therefore, whenever food availability is limited, or energy demands are greater and not associated with compensatory increases in caloric intakes, fertility can be diminished in mammals (Armstrong and Britt, 1987; Berriman and Wade, 1991; Cameron and Nosbisch, 1991; Foster et al., 1995; Zak et al., 1997a), including humans (Cumming et al., 1994; Loucks and Heath, 1994).

Nutritional infertility is particularly common in females due to the great energetic demands of a complete reproductive cycle of ovulation, conception, pregnancy and lactation. This is especially true in those mammals that bear litters. On the other hand, reproduction can be resumed when energetic conditions improve (Wade et al., 1996). As the development of reproductive capacity is essential to the survival of a species, great attention has focused on the onset and maintenance of reproductive activity.

The interactions between nutrition and reproduction have been studied in several species, including rodents (Bronson and Marsteller, 1985; Bronson, 1986;

Murahashi et al., 1996; Wade et al., 1997; Bronson, 1998), swine (Armstrong and Britt, 1987; Cox et al., 1987; Booth, 1990a; Cosgrove et al., 1993; Rozeboom et al., 1993; Zak et al., 1997a,b), cattle (Schillo, 1992; Zurek et al., 1995; Kinder et al., 1995; Bossis et al., 1999; Mackey et al., 1999), sheep (Schillo, 1992; Foster et al., 1995; Martin and Walkden-Brown, 1995), primates (Cameron and Schreihofer, 1995) and humans (Laughlin and Yen, 1996; Loucks, 1996; Laughlin et al., 1998). Particularly in female farm livestock production, where reproductive efficiency is an important economic goal, attempts have been made to understand these interactions, and consequently improve fertility.

In the swine industry, reproductive efficiency is represented by the number of pigs weaned per sow per year, which in turn comprises two main variables: pigs weaned and litters produced per sow per year (Dial et al., 1996). The number of pigs weaned is composed of litter size and pre-weaning mortality, while litters produced per sow per year are dependent on gestation and lactation lengths and on non-productive days (weaning to estrus interval). In turn, litter size derives from ovulation rate, fertilization rate and prenatal mortality (i.e. embryo and fetal mortality). This last factor is responsible for the greater losses in litter size (around 40%), and within prenatal mortality, embryo mortality is reported as an important component (Pope, 1994). The relationship among many known factors affecting embryonic mortality is complex, and the possible mechanisms mediating embryo survival are still being investigated. However, nutrition is certainly an important factor (Ashworth, 1994; Foxcroft, 1997).

Several studies have demonstrated the interactions between nutrition and reproduction in pigs. Armstrong and Britt (1987) reported the cessation of estrous cycles in gilts 46 ± 9 days after they were submitted to chronic, severe energy restriction. However, short-term feed restriction and realimentation in prepubertal gilts has also been established as an effective experimental model for studying the mechanisms mediating nutrition-reproduction interactions (Booth et al., 1994). More recently, Zak et al. (1997a) demonstrated that different patterns of feed intake during lactation, and associated metabolic and endocrine changes, produced differential effects on ovulation rate and embryo survival in primiparous sows. Thus, from a practical perspective, nutrition plays an important role in the reproductive system, as seen when sows and gilts exhibit marked reductions in fertility when subjected to less than optimal nutrition (Kirkwood and Aherne, 1985). From a physiological perspective, considerable progress has been made in the development of experimental paradigms to determine the mechanisms by which nutrition affects fertility (Booth, 1990b; I'Anson et al., 1991; Wade and Schneider, 1992; Cosgrove et al., 1995; Foxcroft et al., 1995; Monget and Martin, 1997; Bronson, 1998; Cunningham et al., 1999; Foster and Nagatani, 1999; Miller et al., 1998).

Gilts are an important component of the breeding herd, and it is essential to improve fertility in these animals, emphasizing genetics, nutrition and management practices. Given the variation in these factors, the development of strategies leading to maximal litter size is challenging. On the other hand, cyclic gilts represent an important experimental model, as their 21-day estrous cycle can be used to represent a 21-day lactation, which is becoming the norm of the swine industry.

Therefore, the purpose of this thesis is to study the mechanisms by which nutrition and metabolic state can affect fertility, and how these mechanisms interact to affect reproductive performance in cyclic gilts. The thesis is composed of five sections: The first presents a review of the literature involving relationships between growth, food intake and metabolism of nutrients (carbohydrates, lipids, proteins, vitamins and minerals), physiology of reproduction, and the mechanisms mediating the interactions between nutrition and reproduction. The next three sections describe three studies using the same experimental paradigm to study nutrition-reproduction interactions in cyclic gilts. The last section will relate the results of all experiments to each other and will include an assessment of the "metabolic theory" in the context of our use of the cyclic gilt model.

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CHAPTER 2

LITERATURE REVIEW

9

Overall, the study of nutrition-reproduction interactions is complex, and these interactions may be mediated by several components. In gilts which are in the process of growth, food intake and metabolism of nutrients play a key role in determining their metabolic state. Additionally, changes in metabolic state may have important implications in reproductive function. Therefore, in this chapter, an attempt is made to describe the key components of nutrition and reproductive function and how they are linked together.

1- Nutritional components

1.1 Growth

The development of a mature phenotype results from an interaction between the genotype of an animal and the environment in which it develops (Foxcroft, 1980). Food availability, ambient temperature and humidity interact to determine an individual's rate of growth, and therefore, these factors determine its rate of reproductive development (Bronson, 1989). Food availability is certainly the most important environmental factor that can affect growth. This was suggested by Williams et al. (1974) in a study with rats, which were allowed to grow normally only until a particular stage of development, and further growth was then inhibited by restricting the amount of food available to them. When the animals were again allowed unlimited access to food, they experienced a rapid "catch-up" growth. The extent of such "catch-up" growth was also demonstrated in pigs in the earlier studies of Widdowson (1973) in which pigs were kept at a weight of 5-10 kg for one, two or three years. The author found that the longer the period of undernutrition, the less complete the "catch-up" growth was, and the fatter the animal became. Additionally, Bikker et al. (1996a) also demonstrated in finishing gilts that realimentation after a period of feed restriction resulted in compensatory gain and increased feed efficiency. They suggested that this extra gain was mainly accounted for by an increase in the contents of the digestive tract and by the increased growth of metabolically active organs (i.e. liver, kidneys and digestive tract). Moreover, Ritacco et al. (1997) reported that runt pigs experience a period of compensatory growth for at least the first two weeks of life, during which they grow very efficiently. However, runts may have differences in their ability to acquire and use energy that affect their continued postnatal development.

According to Shinckel and de Lange (1996), there are four primary parameters required to obtain the estimates of growth that characterize pig genotypes: 1) daily whole-body protein accretion potential, 2) partitioning of energy intake, 3) energy requirements for maintenance, and 4) daily feed intake. The percentage of whole-body protein accretion increases from birth to approximately 45 to 65 kg (as the percentage of water decreases), whereas after 65 kg, the percentage of body protein decreases as the percentage of lipids increases. One of the important factors determining body protein accretion rate is energy intake. Thus, when energy is supplied in excess of that required for the maximum rate of body protein accretion, this "excessive" energy intake will all be used to support fat deposition. Animal activity, maintenance of a constant body temperature, and exposure to disease-causing microorganisms are factors to consider when interpreting energy requirements for maintenance. Consistent with the findings of Schinckel and de Lange (1996), subsequent studies of Bikker et al. (1996b) indicated that protein and lipid deposition increased linearly, while body lipid content had a curvilinear increase with increasing energy intake in finishing gilts from 45 to 85 kg of body weight. Therefore, maximization of protein deposition is accompanied by an increase in lipid content of the body. The increment in protein deposition with increasing energy intake diminished with increasing body weight. Consequently, at similar rates of protein accretion, the lipid deposition increased with increasing body weight. Therefore, finishing pigs become fatter than growing pigs. After feed restriction in the growing period, compensation in protein retention is likely to occur only in the organs. More recently, Möhn and de Lange (1998) suggested that in a stress-free environment and when given adequate intakes of essential nutrients, protein deposition (PD) in growing pigs is determined by either energy intake or the genetically determined upper limit to body protein deposition.

In addition, studies conducted in lambs highlighted the importance of nutrition in altering body composition of neonatal animals and the fact that following prenatal growth restriction there are carry-over effects on energy metabolism and body composition (Greenwood et al., 1998).

The somatotropic axis is a multi-level hormonal system, primarily consisting of growth hormone (GH; somatotropin) and insulin-like growth factor-I (IGF-I; somatomedin), their associated protein carriers and receptors. Circulating GH is a major regulator of metabolism and postnatal longitudinal growth. Furthermore, GH administration elevates plasma insulin and glucose levels (Breier, 1999). IGF-I was originally described as an endocrine factor secreted from the liver in response to GH stimulation, which mediated the effects of GH in peripheral tissues. Treatment with IGF-I stimulates protein metabolism and has insulin-like effects on glucose metabolism. IGF-I reduces plasma glucose as a result of both increased glucose uptake and reduced glucose production. Additionally, IGF-I is also a potent cell survival factor through its role as an inhibitor of programmed cell death (Breier, 1999).

The role of growth hormone (GH) in the control of animal growth is well documented. Its use in animal production has focused on its influence in effecting changes in the partitioning of nutrients between muscle and fat in response to exogenous administration, resulting in improved lean:fat ratios. The anabolic effects of GH on muscle are believed to be largely mediated by IGF-I, while the effects of GH on adipose tissue are thought to be mediated directly by binding to GH receptors on the adipocyte membrane. Studies conducted in cattle (Dawson et al., 1998) indicated that circulating GH and IGF-I concentrations were increased maximally on low and high planes of nutrition, respectively, after GH administration, with insulin concentrations broadly correlating with IGF-I concentrations. Furthermore, it was suggested GH functions as a prime regulator of lipid metabolism, whereas protein metabolism is subjected to a more complex regulatory interaction between GH, IGF-I and insulin.

1.2 Food intake

Stimuli associated with food intake act at the pre- and post-absorptive level to modulate feeding. The oral cavity, stomach and small intestine are the pre-absorptive sites of those stimuli, whereas the liver and the brain appear to be the post-absorptive sites (Scharrer, 1991).

Taste receptors of the oral cavity are mainly involved in food selection, and do not seem to be important in the control of food intake (Scharrer, 1991). On the other hand, food stimuli in the stomach and small intestines are important determinants of meal size, particularly stomach distension and the presence of nutrients in the small intestine (Houpt, 1984). Stomach distension is signaled to the brain via vagal afferents, whereas the release of the hormone cholecystokinin (CCK) appears to be the signal of satiety arising from the small intestine (Scharrer, 1991). Besides CCK, pancreatic glucagon released during food intake also seems to be involved as an endocrine signal in the production of satiety (Geary, 1990). Satiety factors can potentially affect food intake over the course of individual meals but have limited influences on adiposity by themselves. There are other signals, which are not satiety signals per se, that act over long periods of time to suppress food intake by interacting with meal-related stimuli. These signals are presumably proportional to the size of the adipose mass. It is through this interaction between long-term adiposity signals and meal related satiety signals that the control of food intake is integrated into the homeostasis of fat stores (Woods et al., 1998).

At the post-absorptive level, oxidation of circulating nutrients (i.e. glucose, fatty acids) in the liver and brain may be involved in the regulation of feeding. Body fat also seems to influence food intake in rats, as the loss of body fat by food restriction was followed by a transient hyperphagia, while increased adiposity resulted in transient hypophagia (Scharrer, 1991).

Energy homeostasis is controlled through hormones secreted in proportion to body adiposity, including leptin and insulin, and the central nervous system (CNS) targets upon which they act (Schwartz & Seeley, 1997). The CNS targets include those that stimulate food intake and promote weight gain (anabolic pathways), such as the hypothalamic neuropeptide Y (NPY) axis, and those that reduce food intake and promote weight loss (catabolic pathways), such as the hypothalamic melanocortin system. Hormones that are regulated by adipose tissue (insulin and leptin) inhibit central anabolic pathways and stimulate central catabolic pathways (Woods et al., 1998).

Recent studies have demonstrated the existence of a polypeptide hormone, known as OB protein or leptin, synthesized in fat cells, which could provide a link between adipose tissue and the brain. Leptin appears to play a major role in the control of body fat stores through regulation of feeding behavior, metabolism, and body energy balance in rodents, primates and humans (Campfield et al., 1996). In addition, diet-induced weight loss causes leptin concentrations to fall in proportion to the loss of adiposity. During the acute weight loss of fasting, however, leptin concentrations fall much more than expected from the amount of weight loss, suggesting that energy balance reduces the amount of leptin secreted per unit of fat mass (Schwartz and Seeley, 1997). The same authors reported that leptin reduced food intake and body weight when administered directly into the central nervous system, and it also inhibits the neuronal signaling by hypothalamic NPY. The brain is thought to be a primary target for the anorexic effect of leptin, as direct administration of leptin into the CNS potently reduces food intake, and because leptin receptors are expressed in hypothalamic areas important in the control of food intake (Schwartz et al., 1996). It has been suggested that leptin is transported into the CNS by a saturable receptor-mediated process, and the efficiency with which circulating leptin enters the brain is reduced when plasma concentrations are elevated (Banks et al., 1996).

Leptin and insulin share many properties as adiposity signals. Although insulin is secreted from pancreatic beta cells, its circulating concentrations are proportional to adiposity. Insulin also enters the CNS by a receptor-mediated saturable transport process across brain capillary endothelial cells, and insulin receptors are located in the same key hypothalamic areas as leptin receptors (Baskin et al., 1988). Like leptin, insulin reduces food intake and body weight in a dose-dependent manner when administered directly into the CNS (Woods et al., 1996). Both hormones act in part by influencing the efficacy of meal-generated satiety peptides. For example, the effect of CCK to reduce meal size is potentiated by co-administration of either insulin or leptin. In this way, the size of the fat stores can influence daily feeding behavior by modulating sensitivity of the animal to signals generated by eating per se (Woods et al., 1998). The secretion of both leptin and insulin is influenced by the overall amount of fat stores as well as by short-term changes in energy balance, although insulin secretion in rodents is stimulated acutely in response to meals, whereas leptin secretion is not (Boden et al., 1996). Even though the mechanisms controlling leptin synthesis and secretion remain to be clarified, insulin appears to play a key role (Cohen et al., 1996). In contrast to its immediate effect on circulating glucose, insulin's effect on circulating leptin concentrations is delayed for several hours (Dagogo-Jack et al., 1996).

Neuropeptide Y (NPY) is expressed in several areas of the brain, and its synthesis occurs primarily in neurons of the arcuate nucleus, which project to several hypothalamic areas, including the paraventricular nucleus. NPY stimulates food intake, and inhibits sympathetic nervous system outflow, thereby lowering energy expenditure. Moreover, NPY acts in the hypothalamus to alter the peripheral metabolic milieu so as to favor the synthesis and storage of fat (Schwartz and Seeley, 1997). In fasting animals, insulin and leptin inhibit the expression of the NPY gene, whereas glucocorticoids enhance it (Strack et al., 1995). Therefore, during fasting, activation of the hypothalamic NPY pathway is mediated by reduced concentrations of leptin and possibly insulin, coupled with increased glucocorticoid secretion (Schwartz and Seeley, 1997). Further evidence of the effects of glucocorticoid hormones secreted by the adrenal cortex on energy homeostasis as mediated by effects on NPY comes from studies in which administration of glucocorticoids was able to reverse the effect of fasting and increase both food intake and hypothalamic NPY gene expression, which had been previously attenuated by adrenalectomy (Green et al., 1992). Finally, Chavez and coworkers (1997) have demonstrated that glucocorticoids are endogenous antagonists of leptin and insulin in the control of energy homeostasis.

Sex steroids also play an important role in the regulation of appetite and energy metabolism. Progesterone in the presence of estrogen stimulates appetite and promotes weight gain, whereas progesterone alone has no effect on these parameters (Wade and Gray, 1979). Additionally, testosterone stimulates food intake (Hirschberg, 1998). The effects of sex steroids on appetite seem to be centrally mediated. Estrogen may increase the activity of the anoretic agent CRH in the paraventricular nucleus, but it also suppresses NPY abundance and release from the same region.

Of particular interest among the central catabolic systems are the melanocortins, peptides cleaved from the proopiomelanocortin (POMC) precursor polypeptide. In the mammalian forebrain, POMC gene expression is limited to the arcuate nucleus neurons that project to areas that participate in energy homeostasis (such as the paraventricular nucleus). Moreover, these areas of the brain also express melanocortin (MC) receptors (specifically MC3 and MC4 receptors) (Woods et al., 1998). Because the CNS melanocortin system exerts effects opposite to those of NPY, it was anticipated that expression of POMC in the arcuate nucleus would be regulated in a manner opposite to that of NPY. Indeed, fasting has been found to reduce POMC mRNA levels in the arcuate nucleus in mice (Thornton et al., 1997). This response is likely due to the reduced leptin signaling, as the level of POMC mRNA was also reduced in the arcuate nucleus of ob/ob mice and leptin administration to these animals reversed this defect. As leptin receptors are expressed on arcuate nucleus POMC neurons, melanocortin neurons appear to be a target of leptin action (Cheung et al., 1997). Therefore, the effect of leptin on energy homeostasis appears to involve, at least in part, the activation of the hypothalamic melanocortin pathway.

Another hypothalamic neuropeptide pathway that contributes to energy homeostasis, and that is regulated in part by leptin and insulin, is corticotropin releasing hormone (CRH), which is synthesized in paraventricular nucleus neurons. Central administration of CRH reduces food intake and body weight, whereas reduced CRH signaling may contribute to the actions of glucocorticoid hormones to promote weight gain and obesity (Schwartz and Seeley, 1997).

1.3 Metabolism of nutrients

1.3.1 Carbohydrates

A consideration of carbohydrate metabolism must include the metabolism of both those carbohydrates provided in the diet and those formed in the body from noncarbohydrate sources (Beitz and Allen, 1989a). Most dietary carbohydrates are in the form of polysaccharides (starch and in some species cellulose, hemicellulose and the pentosans).

After the digestion and absorption of carbohydrates, the resulting glucose and other monosacharides (fructose, galactose and mannose) are transported to the liver, where they are phosphorylated by glucokinase to yield glucose-6-phosphate. Glucose-6-phosphate is the key intermediate in carbohydrate metabolism. It may be polymerized into glycogen, dephosphorylated to blood glucose or converted to fatty acids via acetyl-CoA. It may undergo degradation by glycolysis and the citric acid cycle to yield ATP energy or by the pentose phosphate pathway to yield pentoses and NADPH. It may be synthesized from pyruvate in a multistep process called gluconeogenesis (Figure 2.1).

The concentration of glucose in the blood is regulated by hormones. Epinephrine prepares the body for increased physical activity by mobilizing blood glucose from glycogen and other precursors. Low blood glucose results in the release of glucagon, which stimulates glucose release from liver glycogen and shifts the fuel metabolism in liver and muscles to fatty acids, sparing glucose to be used by the brain. High blood glucose elicits the release of insulin, which speeds the uptake of glucose by tissues and favors the storage of fuel as glycogen and triacylglycerols.

Carbohydrates in foods differ considerably in their effects on postprandial blood glucose and hormonal responses (Bjork et al., 1994). Therefore, both the amount and source of carbohydrate consumed are important determinants of glucose and insulin responses after a meal (Wolever and Bolognesi, 1996). It has been thought that complex carbohydrates (polysaccharides) could have advantages in improving blood glucose profiles over simple carbohydrates (mono- and disaccharides). However, Hollenbeck and coworkers (1985) demonstrated that when simple sugars were introduced in the diabetic diet as part of natural sources like fruit and milk, blood glucose and serum lipid concentrations were increased compared to a controlled diet with a lower content of simple sugars and a higher content of complex carbohydrates. Thus, it is possible that factors such as cellular structure, degree of gelatinization (hydration of starch during heat treatment), and type of starch may be more important for determining the postprandial blood glucose and insulin responses than molecular size (Vessby, 1994). Additionally, in starchy foods, the structure of the substrate itself affects enzyme availability and hence the glycemic response; i.e. the gelatinization of starch increases its availability to amylases (Bjorck et al., 1994). Furthermore, the content of dietary fiber may play an important role in determining the blood glucose response after a meal by delaying the emptying of the stomach and the passage of the food into the intestine (Smith, 1994; Vessby, 1994).

The glycemic index is a classification of foods based on their blood glucose-raising potential (Wolever, 1997). The glycemic index values mainly reflect how promptly and rapidly glucose enters the blood after food ingestion (Trout et al., 1993), and values can vary markedly, depending on variety, processing and preparation of foods (Wolever, 1997). Thus, the presence of fiber, protein, fat and phytate in foods tend to reduce their glycemic index to some degree (Trout et al., 1993).

Many factors may influence carbohydrate absorption. Nutritional factors that may slow the absorption of starch include dietary fiber, nature of starch (e.g. amylose versus amylopectin and degree of gelatinization), enzyme inhibitors, food form (degree of hydration or particle size) and altered feed frequency, where the nutrients remain the same but the rate of delivery is changed (Jenkins et al., 1994). Reducing the size and increasing the frequency of meals has been shown to result in lower mean blood glucose and insulin over the day (Jenkins and Jenkins, 1995). However, with once a day feeding of a restricted feed allowance, the animal can enter a considerable period of fasting before the next meal, resulting in changes in metabolic hormones that control the switch from increased glycogenesis and decreased glycogenolysis and gluconeogenesis, to the opposite situation of glucose mobilization within a twenty-four hour period (Foxcroft, 1990).

In herbivorous animals, cellulose, hemicellulose and pentosans are converted to short-chain or volatile fatty acids by microbial fermentation in the digestive tract. Acetate, propionate and butyrate fatty acids predominate, whereas propionate is the main glucose precursor in the liver. Their concentrations may vary with time after eating, pH and microbial composition of the ruminal contents. In adult ruminants and nonruminant herbivorous, relatively small amounts of dietary carbohydrates escape fermentation. Because ruminants derive the major portion of their energy from volatile fatty acids, glucose and other monosaccharides play only a secondary role in energy metabolism of these animals (Beitz and Allen, 1989a). However, glucose is the main source of metabolic energy for the ram testis (Martin and Walkden-Brown, 1995).

The brain uses energy for the transmission of impulses and it uses glucose as its main fuel. Glucose is oxidized by the glycolytic pathway and the citric acid cycle, providing almost all of the ATP used by the brain. ATP energy is required to create and maintain an electrical potential across the plasma membrane of neurons (Lehninger, 1993). Furthermore, glucose is rapidly metabolized to glutamate, aspartate, gamma aminobutyric acid (GABA), glutamine and alanine, which are neurally active amino acids and known neurotransmitters (I'Anson et al., 1991).

There is evidence that insulin can act as a modulator of glucose uptake by the brain, as it can cross the blood brain barrier into the cerebrospinal fluid, from where it is taken up by neural tissue in rats (Steffens et al., 1988). This finding is supported by the evidence of the presence of insulin receptors in the central nervous system (CNS), which leads to the concept that the adult brain is an insulin-sensitive organ (Schwartz et al., 1992).

Glucose availability within the CNS may provide signals to the reproductive system by several mechanisms. First, by the direct effect upon the metabolic fuels available to the CNS; second, by causing alterations of metabolic hormones, and providing potential signals to the reproductive axis. Since glucose is the precursor for a number of neurotransmitters within the brain, the degree of neuronal activity may be altered (I'Anson et al., 1991).

1.3.2 Lipids

Triacylglycerols are the most significant group of lipids involved in energy metabolism of animals. They may be provided in the diet or may be synthesized from non-lipid sources in the liver, adipose tissue and lactating mammary gland, as well as in the kidney, brain and lung (Beitz and Allen, 1989b).

Triacylglycerols ingested in the diet are emulsified in the small intestine by bile salts, hydrolysed by intestinal lypases, absorbed by intestinal epithelial cells and reconverted into triacylglycerol. Depending on the length of the carbon chain, fatty acids are released from the enterocytes as lipoproteins (mainly chylomicrons and very low density lipoproteins-VLDL) or as free fatty acids (Booth, 1990 a). Triacylglycerols catabolism to CO_2 is an important generator of useful energy in animals. They are hydrolysed by tissue lipases, and the released fatty acids are oxidized or transported to other tissues as albumin-fatty acid complexes. Liver, heart and resting skeletal muscle rely on oxidation of fatty acids to CO_2 (Lehninger, 1993).

Acetyl CoA is the key component relating carbohydrate and lipid metabolism. The major sources of acetyl CoA are glucose, fatty acids and amino acids. When glucose catabolism is depressed, such as in insulin deficiency and fasting, acetyl CoA oxidation by the citric acid cycle is decreased, diverting acetyl CoA to ketone body production and there is a marked tendency to convert available sources, particularly glucogenic amino acids into blood glucose through gluconeogenesis (Beitz and Allen, 1989b) (Figure 2.2).

Although the brain cannot directly use free fatty acids or lipids from the blood as fuels, it can, when necessary, use 3-β-hydroxybutyrate (a ketone body) formed from fatty acids in hepatocytes. The capacity of the brain to oxidize β-hydroxybutyrate via acetyl CoA becomes important during prolonged fasting or starvation, after essentially all the liver glycogen has been depleted, because it allows the brain to use body fat as a source of energy. The use of β-hydroxybutyrate by the brain during severe starvation also spares muscle protein, which becomes its ultimate source of glucose (via gluconeogenesis) (Lehninger, 1993). In addition, fatty acid oxidation is tightly regulated. High carbohydrate intake suppresses fatty acid oxidation in favor of fatty acid biosynthesis (Beitz and Allen, 1989b).

Circulating lipids may also act as signals of metabolic status to the liver and brain. Receptors in the liver are sensitive to the oxidation of fatty acids, glycerol and 3-β-hydroxybutyrate. Hence, the oxidation of these substrates may alter the activity of hepatic vagal afferent nerves that send information to the CNS. Dietary lipids can also directly alter brain neurotransmitter concentrations, as the blood concentration of choline may determine brain uptake of this substrate, which controls the synthesis of the neurotransmitter acetylcholine (Hirsch and Wurtman, 1978). The increase in blood choline concentrations, and consequently the rise of brain acetylcholine concentrations, could possibly increase the activity of the cholinergic neurotransmitter system (Fernstrom, 1981).

1.3.3 Proteins

Proteins are essential organic constituents of all cells and constitute approximately 18% of the body weight of animals.

Dietary proteins are hydrolyzed in the lumen and in the mucosal cells of the gastrointestinal tract by the action of numerous proteases and peptidases, resulting in production of free amino acids that are transported to the liver via the portal blood (Beitz and Allen, 1989c). Amino acids that enter the liver have several important metabolic routes: they act as precursors for protein synthesis in hepatocytes (liver proteins and most of the plasma proteins); they may pass from the liver into the blood and to the organs to be used as precursors in the synthesis of tissue proteins; certain amino acids are precursors in the biosynthesis of nucleotides, hormones and other nitrogenous compounds in the liver and other tissues; some amino acids may be deaminated and degraded to yield acetyl CoA, via the citric acid cycle. Also, in the period between meals, especially if this period is prolonged, there is some degradation of muscle protein to amino acids. These amino acids donate their amino groups, by transamination, to pyruvate, the product of glycolysis, yielding, in turn, alanine that is transported to the liver and deaminated. The resulting pyruvate is converted into blood glucose by hepatocytes (via gluconeogenesis) and the NH_3 is converted into urea for excretion. The glucose returns to the sketetal muscles to replenish muscle glycogen stores. Additionally, the amino acid deficit incurred in the muscles is made up after the next meal from incoming dietary amino acids (Lehninger, 1993) (Figure 2.3).

As nutrition influences plasma amino acid concentrations and competition occurs between amino acids for brain uptake, nutrition not only influences the provision of substrates for brain protein synthesis, but also controls the activities of substrate-limited neural pathways and brain neurotransmitters (Booth, 1990a).

Amino acids are transported into the brain by one of the several transport systems present in the blood-brain barrier, the acidic, basic and large and small neutral amino acid transport systems. Since amino acids of each class compete with each other for uptake by their respective transport system, the rate of uptake of each amino acid is dependent not only on its plasma concentration, but also on the plasma concentration of its competitors (I'Anson et al., 1991).

Nutrition influences the activities of substrate-limited neural pathways and brain neurotransmitters, as it influences plasma concentrations of amino acids and because competition occurs between amino acids for brain uptake. Furthermore, tryptophan and tyrosine are precursors of the neurotransmitters serotonin and the catecholamines (including dopamine, noradrenaline and adrenaline), whereas histidine and glutamine are precursors of histamine and gamma aminobutyric acid (GABA) neurotransmitters, respectively (Fernstrom, 1981). In general, the consumption of either a protein-containing or carbohydrate-containing meal elevates brain tyrosine concentrations and the rate of synthesis of the neurotransmitters dopamine and noradrenaline. In contrast, brain tryptophan and serotonin concentrations are decreased following protein ingestion, but elevated as a result of carbohydrate consumption.

Therefore, dietary peptides and amino acids have the potential to modulate reproductive status by acting directly as neurotransmitters and neuromodulators or indirectly as precursors to CNS neurotransmitters. They also stimulate a variety of metabolic changes, which provide signals to the reproductive axis (e.g. growth hormone, prolactin, GnRH).

1.3.4 Vitamins

Vitamins are organic compounds that act in small, and in some cases extremely small quantities, and they function as metabolic catalysts usually in the form of coenzymes (Smith and Frey, 1989). In general, all vitamins are required for reproduction because of their cellular roles in reproductive tissues (Ashworth, 1994). Marginal vitamin deficiencies can be manifested as impaired fertility before clinical symptoms of the deficiency are apparent (Hurley and Doarne, 1989).

β-carotene is the major dietary precursor of vitamin A and is believed to have an important role in reproduction distinct from vitamin A (Ashworth, 1994). Akordor and coworkers (1985) have suggested that low β-carotene consumption could increase the occurrence of ovarian cysts and silent estrous periods, as well as delayed conception in lactating cows. Weekly injections of β-carotene in pigs reduced embryo mortality and increased piglet numbers and weight, in the absence of an increase in ovulation rate (Brief and Chew, 1985). More recently, Whaley et al. (1997) reported that vitamin A injections before estrus also restored embryo survival to normal levels in gilts fed high-energy diets and this may be attributable to decreased variation in embryo size. β-carotene forms an integral component of the bovine corpus luteum membrane and progesterone secretion is increased by the addition of β-carotene to bovine and porcine cells *in vitro* (O'Fallon and Chew, 1984; Graves-Hoagland et al., 1988; Talavera and Chew, 1988). In turn, the retinoids are the main metabolites of vitamin A and are involved in cell proliferation and differentiation, expression of growth factors, gene transcription and steroidogenesis (Bagavandoss and Midgley, 1987), which play important roles in embryo survival (Robinson, 1996).

Folic acid is essential for nucleic acid (DNA and RNA) synthesis and its deficiencies are believed to compromize male and female gametogenesis (Wynn, 1987). It has been suggested that folic acid supplementation may prevent deficiencies in DNA synthesis in the early conceptus and Tremblay et al. (1989) observed that the supplementation of folic acid in sow diets was associated with higher serum zinc concentrations in early pregnancy.

In addition, vitamin C is important as a cofactor in steroidogenesis (Ashworth, 1994; Robinson, 1996) and it may, therefore, enhance luteal function (Robinson, 1996).

1.3.5 Minerals

Minerals are inorganic elements that animals require for their normal life processes (Hays and Swenson, 1989). Several minerals have been implicated in influencing reproductive function, especially in terms of affecting embryo survival.

It has been demonstrated that selenium supplementation reduces embryo mortality during implantation in ewes. It also increases fertilization rate, probably by its stimulating effect on uterine contractions and sperm transport (Robinson, 1996). Hidiroglou (1979) suggested that copper deficiency may contribute to embryonic mortality in cattle and dietary molybdenum supplementation decreased conception rate in cattle, associated with a reduced release of LH (Philippo et al., 1987). Phosphorus has been considered the major factor affecting the reproductive performance in dairy cattle (Robinson, 1996), probably due to its participation in the metabolic processes of energy exchange (Hays and Swenson, 1991). Elevated zinc levels during early pregnancy may be beneficial as zinc has been implicated as an activator of steroidogenic enzymes (Hurley and Doarne, 1989).

More recently, chromium has been considered a good example of a mineral having an indirect nutritional effect on the conceptus or on the uterus. Its mechanism of action is to potentiate insulin-mediated effects on the ovary (e.g. increase ovulation rate, improve follicle maturation, increase progesterone production) and on the uterus (e.g. increase uterine production of pregnancy-specific proteins) (Foxcroft, 1997).

2- The reproductive system

The capability to reproduce is essential for the survival of a species, and this capability begins with the attainment of puberty. Reproductive function is coordinated by central (hypothalamus/pituitary) and local (ovarian) events. The physiological mechanisms involved in the coordination of those events, following the attainment of puberty, are the focus of the next section.

2.1 Puberty

Puberty attainment in animals represents the onset of reproductive capability and the achievement of sexual maturation, which is essential to the survival of a species. It is desirable and necessary to achieve the earliest puberty attainment in the gilt, as she is a costly non-productive animal until the initiation of the first pregnancy (Hughes, 1982). Gilts are anatomically ready for reproduction by four to five months of age, but, in general, puberty does not occur before six to seven months of age (Kirkwood et al., 1987).

2.1.1 Factors influencing puberty attainment

Several reviews have considered the relationship between puberty attainment and the age, body weight and growth rate of gilts (Cunningham et al., 1974; den Hartog and van Kempen, 1980; den Hartog and Noordewier, 1984; Knott et al., 1984; Deligeorgis et al., 1985; Beltranena et al, 1991a), yet there is still controversy regarding their importance in the onset of puberty. However, the three factors are intimately related and should be considered together, as they provide information on the stage of development of the gilt. This measure of physiological development (referred to as physiological age) is used to indicate the degree of maturity of the hypothalamic-pituitary-ovarian axis and thus, the ability of the gilt to respond to puberty stimulation (Hughes, 1982).

Body composition as a determinant in puberty onset was first suggested by Frisch and Revelle (1970) as they demonstrated that a minimum percentage of body fat must be reached before the beginning of menarche in humans. The patterns of adipose and lean tissue growth in pigs were reviewed by Schinckel and de Lange (1996), who proposed that as growth proceeds after 90 kg body weight, the rate of lean deposition decreases, whereas the rate of fat deposition may increase. It is possible that the effects of body composition on the attainment of puberty have been confounded by phenotype and genotype. Selection procedures may, therefore, exert important effects on the onset of puberty (Close and Mullan, 1996). As stated by Young et al. (1990), the measurement of fatness may be an indicator of approaching physiological maturity, although a threshold of fatness *per se* may not be a critical prerequisite for puberty onset.

In addition, initial selection for high growth rate may result in the selected population being not only younger at puberty, but also lighter and thus at a lower percentage of their mature body weight (Foxcroft, 1980). Kirkwood and Aherne (1985) have suggested that such differences may be related to rates of tissue deposition, and hence variations in body composition and in mature body size.

A major factor controlling weight at any given age is level of nutrition and there have been several attempts to examine the influence of nutrition on the attainment of puberty in gilts (Close and Mullan, 1996). Den Hartog and van Kempen (1980) reported that feed restriction delayed puberty in 14 trials, advanced it in five trials, but had no effect in eight trials. The response may be dependent upon the degree of feed restriction and it is likely that there is a critical intake below which the animal responds. Severe feed restriction will delay puberty because all somatic growth will be slowed, especially growth associated with the reproductive system, which may have a lower priority for nutrients than other body organs (Close and Mullan, 1996). In addition, it cannot be generally assumed that the effects associated with feeding are attributable to the levels of energy intake *per se*, since differences in feed intake also create imbalances in the intake of amino acids, vitamins and minerals. Although there is limited evidence concerning vitamins and minerals, it has been demonstrated that a reduction in dietary protein will delay the onset of puberty (Cunningham et al., 1974; den Hartorg and van Kempen, 1980).

Furthermore, the continuous selection for lean growth performance appears to result in increasing weight relative to physiological age in gilts fed to appetite. Thus in most gilts, the age at which they are first genetically capable of starting to cycle will be achieved well after they have reached the minimal growth rate or body weight needed to reach sexual maturity (Foxcroft et al., 1996). In the study of Beltranena et al. (1991a), the minimum age at puberty appeared to be approximately 160 days and could be reached with an average growth rate of about 0.60 kg/day from birth to puberty. It is also possible that excessively lean genotypes with very high growth rates may not reach an adequate fat:lean ratio to trigger final sexual maturation and this may actually result in an increase in age at puberty (Foxcroft et al., 1996). Indeed, White and coworkers (1993), using Chinese Meishan and Yorkshire gilts, reported that Chinese Meishan reached puberty much earlier and were in estrus longer than Yorkshire females. However, the possible mechanisms that trigger the early onset of puberty in Meishan pigs deserve further investigation.

Other environmental factors and husbandry and management practices may also be important for inducing puberty at a younger age. Deligeorgis et al. (1984a) demonstrated that full contact with a mature boar is effective in inducing the earliest stage of puberty onset in gilts and the eventual time of response (puberty) is directly related to the maturation of the gilt at the time of boar exposure (Deligeorgis et al., 1984b). The mechanism by which boar contact stimulates puberty attainment in gilts has been investigated by Kirkwood et al. (1981). They demonstrated that boar contact is ineffective in stimulating precocious puberty attainment in gilts that are unable to perceive pheromones. This implies that the "boar effect" is mediated via a primer pheromone. However, other boar stimuli may act synergistically with the primer pheromone(s) to produce the complete "boar effect" (e.g. auditory and visual cues), as proposed by Deligeorgis et al. (1984a).

There is limited evidence in the gilt that "stress" factors may stimulate the early attainment of puberty. The studies in this area have concentrated on the interactions of mixing strange gilts, transportation and relocation with results indicating that all these "management stresses" may have stimulatory effects on the onset of puberty in gilts (see Hughes, 1982 for review). However, boar contact is a more potent form of puberty stimulation than is any form of "stress" although this contact itself may also include a "stress" component (Kirkwood et al., 1981).

2.1.2 Physiological mechanisms controlling sexual maturation in gilts

In comparison to other stages of the adult reproductive cycle, elevated plasma concentrations of both LH and FSH have been consistently recorded in the neonatal period in both males and females (Paterson, Lancaster and Foxcroft, unpublished data). In gilts, neonatal FSH levels are maintained or even exceeded in the first 30 to 50 days of life. Thereafter, a gradual decrease in circulating FSH is reported in the mid prepubertal period (approximately 50 to 150 days), followed by a stabilization of FSH levels in the late prepubertal stages (Foxcroft et al., 1989a). It has been demonstrated that the secretion of LH has a divergent pattern. A decline in plasma LH has been observed after birth and Stickney (1981) reported an increase in plasma LH from mid to late prepuberty, with plasma LH reaching a plateau as puberty approaches; this change is associated with an increase in episodic LH frequency. The same author also observed an increase in FSH secretion in the peripubertal period.

The visible signs of puberty are stimulated by an increase in steroid hormone production by the gonads, in particular estrogen and testosterone. Increased steroidogenic activity of the ovaries and testis in turn is stimulated by increased secretion of the pituitary gonadotropic hormones (LH and FSH). Together, these hormones and the gonadal steroid hormones are responsible for activating and supporting gametogenesis and maturation of the organs of the reproductive tract. These internal processes confer reproductive competence on the maturing individual (Cameron, 1996).

Most investigators feel that the failure to produce a high level of GnRH secretion for a prolonged period is responsible for the relative quiescence of the prepubertal gonad and that this hypogonadotropic condition limits the transition into adulthood. By the time increased GnRH occurs, the pituitary has attained the ability to respond to this stimulus, the ovary has attained the ability to respond to LH and FSH, and the hypothalamo-hypophyseal system has attained the ability to respond to the stimulatory feedback actions of estradiol (Foster et al., 1989). Thus, the increase in LH and FSH secretion at puberty appears to result from a rise in secretory activity of the hypothalamic gonadotropin releasing hormone (GnRH) neurons (Cameron, 1996). This evidence came from studies by Wildt et al. (1980) showing that administration of exogenous GnRH to female monkeys could stimulate increases in reproductive hormone levels and initiative ovulatory menstrual cycles.

The regulation of the tonic release of gonadotropins has been reviewed by Elsaesser (1982) and there appears to be no evidence for negative feedback regulation by endogenous ovarian factors on LH secretion in the gilt before day 80. This lack of negative feedback before day 80 may therefore be related to a lack of ovarian secretory activity *per se* (Foxcroft et al., 1989a). Stickney (1981) reported a concomitant rise in both peripheral LH and estradiol levels in the late prepubertal period, which supports the “gonadostat” concept of pubertal development. This concept was first proposed by Ramirez (1973), who suggested that changes in sensitivity to the negative feedback of estrogens on the hypothalamus could explain the changes in episodic LH secretion seen at the time of puberty. Furthermore, continuous maturation of the positive feedback mechanism, and an apparent continued dependence on ovarian estrogen to maintain the functionality of this mechanism, appear to be important components of sexual maturation in the gilt (Foxcroft et al., 1989a). More recently, Elsaesser et al. (1998), studying the role of ovarian secretions in modulating positive estrogen feedback on LH release in late prepubertal and sexually mature gilts, reported that continuous ovarian secretion is necessary for the final maturation of the LH surge mechanism in late prepubertal gilts and also for maintaining the full functionality of this mechanism in sexually mature gilts. The range over which ovarian estrogens tune the final maturation of this process appears to be rather narrow, and other ovarian factors may contribute to the final maturation.

Another important aspect of hypothalamic-pituitary maturation has been the study of ovarian-dependent and ovarian-independent regulation of gonadotropin secretion during sexual maturation. The fact that characteristic changes in the pattern of LH secretion occur in ovariectomized females in several species suggests that there are centrally mediated maturational changes that are not dependent on changes in steroid feedback. These central mechanisms may also mediate the actions of environmental factors, such as photoperiod, pheromones and nutrition, which are known to affect puberty onset (Foxcroft, 1993).

Studies of Booth and coworkers (1994, 1996) demonstrated that the immediate metabolic state of the animal, rather than any long term assessment of body weight change or body composition, is the important regulator of hypothalamic-pituitary function. Furthermore, they also showed that an elevation in plasma glucose would mimic the effect of total feed replacement and the insulin-glucose axis is at least partly involved in mediating nutritional effects on sexual maturation. Therefore, if high planes of nutrition are made available, then growth rate and body composition are unlikely to limit the attainment of puberty (Foxcroft, 1993). Interestingly, Booth et al. (1996) also presented clear evidence for a nocturnal increase in episodic LH secretion in feed restricted gilts of around 80 kg body weight, analogous to the nocturnal rise in LH secretion reported in pubertal boys by Faiman and Winter (1974).

2.2 Endocrinology of the estrous cycle

2.2.1 The luteal phase

The luteal phase is characterized by high levels of progesterone in peripheral plasma and low episodic LH release (low frequency, high amplitude), with progesterone acting as the major component of ovarian negative feedback on gonadotropin secretion. Generally, the principle modulator effect of elevated progesterone secretion is to cause a decrease in the frequency and an increase in the amplitude of episodic LH secretion (one large episode every three hours) with little effect on FSH release (Foxcroft, 1993). High concentrations of progesterone also inhibit the estrogen positive feedback mechanism, thereby ensuring that the ovulatory process *per se* is not operative during established luteal function. Furthermore, the absence of follicular growth in this phase is responsible for the low peripheral estrogen concentrations.

There is increasing evidence that the signals for initiation of recruitment of follicles into the follicular phase of the cycle are coincident with the onset of luteolysis (Foxcroft, 1993). In pigs, luteolysis occurs around days 14-15 of the estrous cycle (considering day 0 the first day of standing heat), and is mediated by the release of prostaglandins ($PGF_{2\alpha}$) from the uterus. Corpora lutea of the cycling pig are generally considered to be resistant to $PGF_{2\alpha}$-induced luteolysis prior to day 12 of the estrous cycle. However, Estill et al. (1993) demonstrated that repeated administration of $PGF_{2\alpha}$ before day 12 caused premature luteolysis and shortening of the estrous cycle by one or two days.

In cattle and sheep, luteolysis occurs as a result of the release of luteolytic episodes of uterine $PGF_{2\alpha}$ in response to the binding of oxytocin to newly developed endometrial oxytocin receptors (Silvia et al., 1991; Wathes and Lamming, 1995). The role of progesterone in the luteal phase is to inhibit the development of the endometrial oxytocin receptor until the appropriate time, while at the same time inducing endometrial ability to synthesize and release $PGF_{2\alpha}$ in response to oxytocin (Lamming and Mann, 1995). Mann et al. (1998) reported that premature exposure to progesterone advances the time at which the ability of progesterone to inhibit the appearance of endometrial oxytocin receptors is lost. Therefore, it would seem more likely that the influence of progesterone on the timing of luteolysis is mediated through the control of the oxytocin receptor.

The use of $PGF_{2\alpha}$ injections to control unproductive days in the swine industry has been investigated. Recently, Armstrong et al. (1999) demonstrated in sows that it was possible to induce ovulation immediately after farrowing using a single injection of human chorionic gonadotropin. However, injection of $PGF_{2\alpha}$ to stimulate luteolysis of the induced corpora lutea and control weaning-to-estrus interval did not seem effective. It is likely that the proper dosage and timing of $PGF_{2\alpha}$ administration must be elucidated before this strategy will benefit the industry.

2.2.2 The follicular phase

Declining progesterone in the peripheral plasma after luteolysis allows an increase in the episodic release of LH (eventually to one pulse per hour or greater), which in concert with FSH, stimulates follicular growth and estrogen synthesis (Foxcroft, 1993).

LH and FSH release from the anterior pituitary is influenced by hypothalamic GnRH. There is evidence that GnRH, secreted in a pulsatile manner from neurons located in the hypothalamus, is responsible for an episodic pattern of LH secretion into peripheral blood (Britt et al., 1985). Studies have demonstrated that hypophysectomy, hypophysial-stalk transection, active and passive immunization against GnRH, and active immunization against LH impair reproduction by interfering with normal follicular development in the female (see Esbenshade et al., 1990 for review). Indeed, Esbenshade and Britt (1985) reported that active immunization of sexually mature gilts against GnRH induced acyclicity, suppressed concentrations of gonadotropins and estradiol, and caused atrophy of the ovaries. Moreover, ovaries taken from gilts actively immunized against GnRH contained few antral follicles and over 98% of follicles had more than four layers of atretic granulosa cells (Esbenshade, 1987). FSH is essential to the development of a preovulatory follicle pool in the porcine ovary, whereas the pulsatile pattern of LH secretion is essential to the final stages of follicular maturation and ovulation of mature oocytes (Foxcroft et al., 1994). Recent studies of mouse follicles *in vitro* (Spears et al., 1998) demonstrated that FSH is essential for follicular growth: in the absence of FSH follicles become clearly atretic, and LH has little role beyond increasing the production of estradiol, until ovulation. Moreover, gonadotropic stimulation of the ovary may increase LH and FSH receptors on theca and granulosa cells and estrogen synthesis is increased.

Estradiol is a highly potent inhibitor of FSH release, apparently by decreasing synthesis through suppression of mRNA for both the α and β subunits of FSH (McNeilly, 1988). There is also evidence that at least two non-steroidal inhibitors of FSH secretion (inhibin and follistatin) are produced by the follicle and, together with increasing estradiol production, are responsible for the gradual inhibition of FSH secretion during the early follicular phase in domestic species (Foxcroft, 1993). Recent studies with guinea pigs (Shi et al., 1999) showed that plasma inhibin started to increase during the early follicular phase, whereas plasma estradiol clearly increased during the late follicular phase. Therefore, it was suggested that inhibin is a chemical signal of the number of growing follicles in the ovary and estradiol is a signal of follicular maturation in the ovary.

With continuous follicular growth, both LH and FSH secretions decline and plasma levels of estradiol increase during the follicular phase of the cycle (Ziecik et al., 1987; McNeilly, 1988). As follicles reach their full development and circulating estradiol levels peak, the feedback effects of estradiol on the hypothalamus and pituitary become stimulatory rather than inhibitory, by stimulating secretory activity of GnRH neurons (Ziecik et al., 1988). As a result of the positive feedback effects of estradiol, there is a surge in GnRH release, and consequently in LH and FSH secretion (Foxcroft and van de Wiel, 1982). The surge of LH stimulates final maturation of follicles and triggers their ovulation 36-40 hours later (Elsaesser et al., 1998).

Knowledge of estrus symptoms and hormonal profiles in the peri-estrus period is important to achieve high fertility at breeding. Studies conducted in gilts and primiparous sows (Blair et al., 1994), as well as in multiparous sows (Mburu et al., 1995; Soede et al., 1994), have associated hormone profiles in the peri-estrus period with embryo survival. It has been suggested that a closer synchrony between the onset of the LH surge and estrus and the peak concentration of estradiol might be associated with an increase in embryo survival and a decrease in embryo diversity. Indeed, studies comparing endocrine profiles of Chinese Meishan, generally characterized by sexual precocity and high prolificacy, and Large-White hybrid gilts (Hunter et al., 1993) demonstrated that the time intervals from the estradiol peak concentration and the onset of the LH surge until the onset of behavioral estrus were different for the breeds, with estrus occurring earlier in the Meishan gilts.

2.3 Ovarian Physiology

Ovarian folliculogenesis is a dynamic process characterized by a marked proliferation of the somatic cell components of the follicle. The developing follicle provides the environment for the maturation of the oocyte in preparation for its fertilization after ovulation (Armstrong and Webb, 1997). Thus, folliculogenesis is a key component of female reproductive function and the dynamics of folliculogenesis will also impact final ovulation rate (Foxcroft, 1993).

The whole process of follicular growth, oocyte maturation and luteinization involves complex interactions between the different follicular compartments and is regulated by endocrine, paracrine and autocrine mechanisms (Foxcroft et al., 1994). The primary regulators of folliculogenesis are the gonadotropins, which regulate ovarian follicular development via classic endocrine mechanisms (Foxcroft et al., 1989b; Grant et al., 1989). Such evidence has developed from studies with prepubertal gilts treated with exogenous gonadotropins (Christenson et al., 1985; Bolamba et al., 1996).

In the adult ovary, folliculogenesis starts when primordial follicles leave the pool of resting stage follicles, being activated by some signal(s) to enter the growth phase. The effects of androgens on ovarian development have been known for some time, but they were often considered to be due to indirect stimulation, via estrogen (Hillier and Ross, 1979). However, recent studies have shown that androgens exert a direct, stimulatory role on the growth and development of mouse antral follicles *in vitro* (Murray et al., 1998). In primates androgens have been shown to promote primordial follicle development through the activation of oocyte IGF-I signaling (increased expression of both the peptide and its receptors in oocytes) (Vendola et al., 1999). The emergence of follicles from the resting stage is generally considered to be gonadotropin-independent. From there, the early growing follicle undergoes a developmental process, including cellular proliferation and differentiation through primary, secondary, antral and finally Graafian stages of development. At least these later stages appear to be FSH-dependent (Gougeon, 1996). Following antrum formation, a follicle has the potential to reach ovulatory status during the span of one estrous cycle. Since it appears that antrum formation of follicles destined to ovulate may occur at the beginning of the estrous cycle, changes in gonadotropins around the time of ovulation may play a role in this event (Morbeck et al., 1992). The timing of the initial secretion of estradiol may be an important indicator for a normally developing preantral follicle and production of large quantities of estradiol may support a normal transition through antral development into the preovulatory stage (Fehrenbach et al., 1998). In studies conducted with hyperprolific sows, Driancourt and Terqui (1996) reported an altered pattern of growth of the ovulatory follicles throughout the follicular phase and increased aromatizing ability of large ovulatory follicles compared to follicles of Large-White sows. This estrogen produced by large follicles may promote growth of smaller follicles. Consistent with these findings, Miller et al., (1998) demonstrated that Meishan sows with a high ovulation rate maintained a large number of follicles during the follicular phase, whereas in Large-White hybrid animals, follicle numbers decreased between days 16 and 19. Preovulatory follicles from Meishan sows also produced more estradiol in vitro than those from Large-White hybrids.

According to Goodman and Hodgen (1983), the later stages of follicular development involve three interacting processes: recruitment, selection and dominance. This complex process induces Graafian follicles within the 'recruitable pool' to enter the final stage of preovulatory development and a variable number of these selected follicles will ovulate. This pattern of late follicle development is associated with changes in expression of mRNA encoding gonadotropin receptors and steroidogenic enzymes and allows selected follicles, when exposed to the requisite hormonal environment, to ovulate in response to the preovulatory gonadotropin surge (Armstrong and Webb, 1997).

2.3.1 Follicle recruitment

Follicular recruitment is the stage during which a pool of growing and resting stage follicles, otherwise destined to become atretic, begin a further and rapid stage of development (Goodman and Hodgen, 1983). Recruitment occurs from a pool of antral follicles ranging from 1 to 6 mm in size. Studies indicate that: a) a continual process of growth and atresia occurs within this proliferating pool, b) the rate of atresia is highest in the smaller sized follicles, and c) only a small proportion of follicles from the total population will be represented in the largest size categories. Therefore, a process of selective atresia already acts to create a population of larger follicles available for recruitment into the follicular phase of growth (dominant follicles). In some species (e.g. rats, primates, pigs), dominant follicles develop only during the follicular phase and are thus destined for ovulation. In other species (e.g. cattle, sheep, horses), recruitment, selection and dominance occur at regular intervals, but only the dominant follicle present during the follicular phase ovulates. There is evidence that the mechanism that allows some follicles to be recruited for potential dominance/ovulation is a small elevation in basal FSH that, by chance, occurs around the time the follicle would normally begin atresia (Fortune, 1994). If recruitment is absent, then atresia of these larger follicles is inevitable and they will be replaced from smaller, non-atretic follicles within the proliferating pool (Foxcroft et al., 1989b). It is likely that both endogenous FSH and

LH are necessary for recruitment and FSH probably sensitizes the follicles to LH (Foxcroft et al., 1994). Gonadotropins act on follicular cells by coupling with receptors localized at the cell surface. FSH binds exclusively to its receptors in granulosa cells, whereas LH binds to LH receptors initially localized to theca interna cells and later to receptors on granulosa cells during maturation of the preovulatory follicle (Gougeon, 1996).

The recruitment of follicles occurs between days 14 and 16 of the porcine estrous cycle and coincides with luteolysis of the corpora lutea (Hunter and Wiesak, 1990). In addition, because the size of the proliferating pool of follicles may be an important determinant of ovulation rate, factors that affect the size of this pool are of practical significance. Genotype and nutrition have been clearly implicated (Dailey et al., 1972; Clark et al., 1973; Dailey et al., 1975; Hunter et al., 1993).

In cattle, the estrous cycle is characterized by a series of follicular waves. During each follicular wave, a group (approximately 5 to 7) of gonadotropin-responsive antral follicles (3 to 5 mm) are stimulated to initiate increased growth. In heifers similarly to gilts, the recruitment of ovarian follicles may also be enhanced by increased dietary intake (Gutierrez et al., 1997). A dominant follicle emerges from this group and, as it continues to grow, it secretes increased amounts of estradiol, inhibin and other factors. The increase in secretions from the dominant follicle causes the remaining subordinate follicles to become atretic and regress. In the presence of a corpus luteum, the dominant follicle remains functional for several days after achieving its maximum size, but then undergoes atresia. The loss of functionality of the dominant follicle results in a transient rise in plasma FSH, which stimulates the emergence of a new follicular wave (Bartlewski et al., 1998). This pattern of growth in follicular waves is repeated until a dominant follicle is present, coincident with regression of the corpus luteum, which marks the beginning of the follicular phase of the cycle and continued growth of that dominant follicle to a preovulatory stage. A follicular wave has a typical duration of approximately 8 to 10 days and the number of follicular waves that occur during the estrous cycle is dependent on the life-span of the corpus luteum. From two to four follicular waves can occur during an estrous cycle, with approximately 75% of cycles comprising three waves (see D'Occhio, 1999 for review). Moreover, the duration of dominance of the ovulatory follicle is associated with fertility in heifers: a dominance period of four days or less results in a precise onset of estrus and a high pregnancy rate (Austin et al., 1999), whereas a dominance period greater than nine days before the gonadotropin surge is associated with a reduction in dominant follicle health (Mihm et al., 1999).

Ovarian follicles are known to produce a range of locally acting peptide/protein growth factors. These growth factors can either interact directly with the same cell type from which they are produced (autocrine action) or with other cell types within the developing follicle (paracrine action) to stimulate or attenuate the cellular response to gonadotropins. Additional mechanisms, such as juxtacrine (activation of receptors on adjacent cells by membrane bound growth factors) and intracrine (stimulation of the cell in which the factor is produced without prior secretion of the factor from the cell) are also recognized as mechanisms through which granulosa and theca cells can control their behavior (Gougeon, 1996; Armstrong and Webb, 1997). These intraovarian peptides are known as regulators of follicular responsiveness to gonadotropins and four groups have been reported as potent regulatory factors: insulin-like growth factors (IGFs), transforming growth factor β (TGF-β), fibroblast growth factors (FGFs) and epidermal growth factor (EGF)/transforming growth factor α (TGF-α) (Cosgrove and Foxcroft, 1996; Armstrong and Webb, 1997).

Insulin-like growth factors (IGFs) function as modulators of gonadotropin action at the cellular level and stimulate granulosa and theca cell proliferation and differentiation. They show distinct species-specific patterns of expression in follicular tissue (Armstrong and Webb, 1997). In swine, the IGF-I gene is expressed in the liver and adipose tissue (Simmen et al., 1992), skeletal and cardiac muscle (Leaman et al., 1990) and uterus (Tavakkol et al., 1992). Charlton and coworkers (1993 a) first reported IGF-I gene expression in porcine ovarian tissue *in vivo*, supporting the hypothesis that IGF-I is a locally produced growth factor that may have autocrine and/or paracrine activity within the ovary. In addition, it was demonstrated that IGF-I is produced by granulosa cells and its synthesis is stimulated by factors such as FSH, GH and estradiol (Adashi et al., 1985; Guthrie et al., 1995; Samaras et al., 1996). Changes in concentration of IGF-I and expression of mRNA encoding IGF-I in the follicular fluid during the follicular phase in pigs indicate that IGF-I may be involved in or linked to the process of selection and growth of ovulatory follicles (Hammond et al., 1993). The most potent ovarian action of IGF-I is apparent as interactions with gonadotropins and other growth factors (e.g. enhancing steroidogenesis and TGFβ-stimulated cellular proliferation and differentiation) (Cosgrove and Foxcroft, 1996; Schams et al., 1999). Interestingly, Killen et al. (1992) reported that stimulation of ovarian steroidogenesis in sows occurs by 6 hours after weaning and that IGF-I is not a primary stimulus for estradiol or progesterone production during the first 24 hours after weaning. In contrast to swine, Perks and coworkers (1999) reported that IGF-I and IGF-II play a significant role in follicular and luteal development in the bovine ovary. Locally produced IGF-II is probably an important regulator of follicular growth, whereas most of the IGF-I present in follicular fluid is likely to be derived from the circulation.

Actions of IGFs are modified by IGF-binding proteins (IGFBPs) because the IGFBPs can block IGF action or, alternatively, enhance it by slowing IGF clearance (Yuan et al., 1996). IGFBPs, present in follicular fluid, are produced by granulosa cells and are stimulated by IGF-I and reduced by FSH (Guthrie et al., 1995). Furthermore, IGFBP activity has been demonstrated to be higher in atretic follicles, suggesting that modulation of IGF-I plays a role in folliculogenesis and follicular atresia (Edwards et al., 1996). Additionally, Howard and Ford (1992) demonstrated that IGFBP-2 may be an important local regulator of follicular steroidogenesis, possibly affecting follicular dominance and atresia.

The TGFβ superfamily is composed of a number of proteins with the potential to act as intraovarian regulators, as demonstrated by the inhibition of granulosa and theca cell proliferation, while gonadotropin-stimulated steroidogenesis was enhanced in cows (Roberts and Skinner, 1991). In contrast to IGFs, TGFβs are released from cells in a latent form that does not bind to its receptor. Hence, the critical step in the regulation of TGFβ activity appears to be its conversion into the active form (Harpel et al., 1992). It is likely that proteolysis provides a physiological mechanism for the activation of latent TGFβ, thus the regulation of TGFβ activity is controlled by the activity of proteases that release mature TGFβ from its latent complex. Furthermore, the activation of TGFβ would be expected to influence the development of dominance, as it acts synergistically with gonadotropins to control the differentiation of follicular cells (Armstrong and Webb, 1997).

Members of the TGFβ superfamily include activins and inhibins. In addition to the well known endocrine role of inhibin in controlling FSH secretion, there is increasing evidence of a role of these proteins as local regulators of ovarian function. Activin appears to play a folliculogenic role in the early stages of follicle development, perhaps by being involved in the selection process, whereas in the later stages, activin might impede follicle growth and differentiation. Moreover, once follicles are selected, activin production may decrease as inhibin levels increase. Inhibin may then promote further development of the follicle to the preovulatory stage since it has the ability to increase thecal androgen production, thereby increasing the substrate for granulosa cell estrogen production (DePaolo, 1997). Follistatin is a binding protein present in follicular fluid that regulates activin activities, as it can bind to cell surface proteoglycans, thus providing an effective and rapid means of regulating activin bioactivity (Gougeon, 1996; DePaolo, 1997). Recently, Ford and Howard (1997) demonstrated that activin depresses *in vitro* steroidogenesis by granulosa cells from antral porcine follicles and, therefore, activin may participate in atresia of antral follicles in the pig. In addition, through its binding of activin, follistatin could potentially interfere with atresia of antral porcine follicles.

Epidermal growth factor (EGF) and its structural homologue TGFα are negative modulators of FSH-induced synthesis of estradiol and positive modulators of granulosa cell proliferation in human follicles (Gougeon, 1996). Singh and Armstrong (1995) provided evidence that TGFα is synthesized by the cumulus and granulosa cells of the porcine ovary, and it is a potent stimulant of porcine oocyte maturation and cumulus expansion, which leads to the release of fertilizable oocytes. Additionally, Ding and Foxcroft (1994b) previously reported that EGF can stimulate nuclear maturation in pig oocytes and it can interact with gonadotropins to enhance cytoplasmic maturation.

The fibroblast growth factors (FGFs) are a family of heparin-binding growth factors, whose effects are mediated by tyrosine kinase receptors. Within the ovary, the most studied member of this family is FGF-2 (Armstrong and Webb, 1997). As detected in rodent follicles, FGF-2 inhibits FSH-stimulated receptor expression in granulosa cells and reduces specific binding of IGFs in thecal tissue (Wordinger et al., 1993). However, further investigation is necessary to clearly elucidate the role of FGFs during folliculogenesis.

2.3.2 Follicle selection

Once recruitment has occurred, a population of follicles is "selected" for further growth. During the selection process, some follicles complete enlargement (become dominant), while others undergo atresia (Foxcroft et al., 1994). According to Foxcroft and Hunter (1985), the growth of selected preovulatory follicles in the pig is associated with rapid atresia of smaller follicles. Furthermore, it has been suggested that in polyovulatory species like the pig the first "dominant" follicles to emerge in the preovulatory population could promote the maturation of the smaller follicles in the recruited pool. Thus, the pattern of follicle growth during the mid- to late luteal phase in pigs (days 7 to 15) may be characterized as continual growth and atresia (Guthrie and Cooper, 1996). In a study by Grant and coworkers (1989) on follicle development in cyclic gilts on days 16, 18, 20 and 21, the progressive decrease in follicle number per animal from day 16 to 21supported the previous suggestion of Clark et al. (1973) that during the follicular phase there is a physiological block to the replacement of atretic follicles in the proliferating pool. This decrease in number was accompanied by an increase in the size of the follicles present and by day 21, all the follicles present were greater than 8 mm in diameter. Additionally, the selection of the preovulatory population continued over the 4-day period from about day 16 to day 20 of the cycle (Grant et al., 1989).

Gonadotropins are known to affect follicular cell differentiation. The pattern of LH secretion may play an active role in preovulatory follicle selection by altering the responsiveness of the growing follicle to FSH and may act in concert with the decline in FSH secretion to enhance atresia in follicles deprived of FSH (Fortune, 1994). Moreover, during the follicular phase in vivo, FSH induces maturational changes and stimulates estradiol production by follicular cells, resulting in the release of the preovulatory surge of LH (Ding and Foxcroft, 1994a).

On the other hand, estradiol plays an essential role in mammalian folliculogenesis. In most species, a rise in estradiol synthesis and secretion immediately precedes the preovulatory surge of gonadotropins (Conley et al., 1994). Steroidogenesis during follicular maturation is a complex process involving the interaction of two follicular compartments (granulosa and theca cells) and the action of gonadotropins and intragonadal regulators (Yuan et al., 1996). Estrogen synthesis is dependent on the availability of androgen and the conversion of androgens into estrogens. Androgens synthesis occurs through the activity of the enzyme cytochrome P450 17αhydroxylase/17-20 lyase ($P450_{c17}$) and the conversion of androgens into estrogens is catalysed by cytochrome P450 aromatase ($P450\text{-}_{arom}$) (Conley et al., 1994). More importantly, each of the follicular tissue compartments (granulosa and theca cells) responds to gonadotropic stimulation by the synthesis of different steroid products. In general, granulosa cells can convert androgens to estrogens but are incapable of androgen synthesis, which occurs exclusively in the theca. However, the theca interna of pigs demonstrates $P450\text{-}_{arom}$ activity in addition to $P450_{c17}$. Therefore, porcine theca can synthesize estrogen independently of the granulosa (Evans et al., 1981). FSH and LH are the principle promoting agents responsible for steroidogenesis, synthesis of steroidogenic enzymes mRNA (e.g. $P450\text{-}_{arom}$, $P450_{c17}$) and the increase in the number of steroidogenic cells, as a consequence of stimulation of follicular growth (Shoham and Schacater, 1996). Garret and Guthrie (1996) demonstrated that increased expression of steroidogenic enzymes was associated with follicular growth and that loss of $P450\text{-}_{arom}$ expression *in vivo* is an early event in atresia, followed by decreased cell proliferation and decreased expression of $P450_{c17}$.

Furthermore, Yada et al. (1999) reported that granulosa-theca cell interaction is a major follicular constituent in the control of follicular cell differentiation and proliferation during ovarian follicular development. The theca factor(s) inhibits the differentiation of granulosa cell at the early stage of follicular development, but promotes differentiation during late follicular maturation. On the other hand, granulosa factor(s) promotes both differentiation and growth of theca cells throughout the follicular maturation process. Therefore, the cross-talk between granulosa and theca cells is essential for the maintenance of the physiologic function and structure of follicular cells.

2.3.3 Follicular heterogeneity

. Grant and coworkers (1989) demonstrated by the range of morphological and biochemical developments on day 16 that not all follicles destined to ovulate are at the same stage of maturity at the time of recruitment and selection. This asynchrony (heterogeneity) was evidenced by the marked differences in steroid synthesizing ability of follicles of identical size within the same ovary. A further consequence of the asynchrony in the rate at which follicles become estrogenically active is the period of time follicles are in a "mature" state before the onset of the preovulatory LH surge. Probably the high estrogenic activity of the dominant follicles provides the trigger to the preovulatory LH surge and thus sets the timing of the surge and of ovulation. Therefore, at the time of the LH surge and subsequent ovulation, follicles may be at different stages of morphological and biochemical maturation, with implications for oocyte maturation and early embryonic development (Hunter and Wiesak, 1990).

2.3.4 Oocyte maturation

The onset of meiosis in mammalian oocytes occurs during prenatal life after a period of high mitotic activity. However, when the dictyate (germinal vesicle) stage is reached, the first meiotic division is arrested during further fetal and neonatal development. Once animals reach sexual maturation, fully grown oocytes contained within the recruitable pool of follicles can re-enter meiosis in response to the preovulatory LH surge and re-activate cytoplasmic programming. This re-activation will then yield mature oocytes that have the ability to be fertilized and to undergo subsequent embryonic development. This process, which transforms a primary oocyte into an unfertilized egg, is called oocyte maturation (Shoham and Schachter, 1996) and both nuclear and cytoplasmic changes are involved (Foxcroft et al., 1994).

Nuclear changes begin with chromatin condensation and nuclear membrane disintegration (germinal vesicle breakdown, GVB) and progress through metaphase I to metaphase II (the completion of the first meiotic division). GVB is the first microscopical sign of oocyte maturation and can be taken as evidence for the resumption of meiosis. The meiotic process continues until the subsequent extrusion of the first polar body containing half of the number of chromosomes, and formation of the second meiotic spindle (Tornell et al., 1991; Shoham and Schachter, 1996). Completion of first meiosis is one of the prerequisites for oocytes to be fertilized and undergo embryonic development (Foxcroft et al., 1994). In pigs and cows, meiotic competence is closely linked to final growth of the oocyte and occurs in comparatively larger sized follicles (Leibfried-Rutledge et al., 1989).

The preovulatory LH surge is the normal signal for the resumption of meiosis. LH has receptors on the granulosa and theca interna cells, but not on the oocyte, and its action on the oocyte is therefore mediated indirectly via the granulosa cells (Tornell et al., 1991; Shoham and Schachter, 1996). On the other hand, a number of factors, such as oocyte maturation inhibitor (OMI) and purine nucleosides found in the follicular fluid, show inhibitory effects on oocyte maturation *in vitro* (Tornell et al., 1991). For example, cyclic adenosine monophosphate (cAMP) prevents oocyte maturation *in vitro*, thus the decline in oocyte levels of cAMP can be causally related to the commitment period and occurs as one of the earliest events after induction of maturation. There is some controversy concerning the mechanism by which cAMP levels are maintained within mammalian oocytes. It has been suggested that the cumulus may transit cAMP, via gap junctions, to the mammalian oocyte to maintain meiotic arrest (Leibfried-Rutledge et al., 1989). Moreover, according to Osborn and Moor (1983) a precise balance of steroids is necessary for the full maturation of mammalian oocytes. Estrogen is probably the major steroidal signal during the critical early stage of maturation but is probably replaced by androgen or one of the progestagens in the later phases. The mechanism underlying such steroidogenic control remains to be determined (Racowsky, 1993).

In addition to the process of nuclear maturation, the cytoplasm of the oocyte must undergo some critical changes. Cytoplasmic maturation in pig oocytes is dependent on follicular cell support and steroids secreted by follicular cells are involved in the regulation of cytoplasmic maturation (Mattioli et al., 1988). Other studies suggest the involvement of non-steroidal factors (Ding and Foxcroft, 1994a). Furthermore, the presence of follicular cells delays the uncoupling between cumulus cells and oocytes. This prolonged intercellular coupling may be essential for oocytes to obtain sufficient nutrients and bioactive molecules from cumulus cells or the surrounding environment (Foxcroft et al., 1994).

Gonadotropins are the primary regulators of nuclear maturation of mammalian oocytes *in vitro*. However, other factors, including growth factors, also appear to regulate ovarian function, as described in rodents (Downs, 1989), cows (Lorenzo et al., 1994) and pigs (Ding and Foxcroft, 1994b). Downs (1989) reported stimulation of oocyte maturation and cumulus expansion *in vitro* by epidermal growth factor (EGF); EGF appears to promote oocyte maturation by disrupting the communication between cumulus and oocyte. TGFα also stimulates the mitotic maturation of cumulus cells associated with mouse oocytes (Brucker et al., 1991). TGFβ stimulates the maturation of oocytes in rats (Feng et al., 1988) and IGF-1 enhances nuclear maturation in oocytes surrounded by compact cumulus cells in bovine (Lorenzo et al., 1994). Totey and coworkers (1996) demonstrated that insulin and IGF-1 can both stimulate buffalo oocyte maturation and their effects are regulated by FSH. They also demonstrated that the transcripts encoding for insulin and IGF-1 ligands and receptors were observed on cumulus cells and granulosa cells and can act through autocrine and paracrine mechanisms.

2.3.5 The corpus luteum

The process of luteinization is triggered by the preovulatory gonadotropin surge and establishes a remarkable remodeling of the ovary. The most notable functional change is the shift from androgen and estrogen production to synthesis of large quantities of progesterone (Smith et al., 1994). To do so, granulosa cells, which transform androgens from the theca into estrogens, acquire the competence for the entire process of progesterone synthesis, i.e. the capability to employ cholesterol as substrate. Paracrine growth factors (EGF, IGF-1) modulate both differentiation and proliferation, processes that appear to be mutually exclusive in luteinizing granulosa cells (Pescador et al., 1999).

The mobilization and delivery of cholesterol to the mitochondria is ligand-dependent in ovarian steroidogenic cells. A key step in the process is the transport of cholesterol from intracellular stores to the inner mitochondrial membrane, where it is transformed to pregnenolone by cytochrome P450 side-chain cleavage enzyme ($P450_{scc}$). This transfer can be affected by a protein, steroidogenic acute regulatory protein (StAR), expression of which is stimulated by FSH and cAMP (Pescador et al., 1999). Moreover, pregnenolone is converted into progesterone by the enzyme 3β hydroxysteroid dehydrogenase (3β HSD) in the lysosome.

The corpora lutea of pigs contain small and large luteal cells, which are regulated in different ways by LH and prostaglandin E_2 (PGE_2). According to Richards et al. (1994), LH was found to stimulate progesterone secretion by small but not by large luteal cells. Conversely, PGE_2 stimulates progesterone secretion by large but not by small luteal cells (Richards et al., 1994). Therefore, regulation of progesterone secretion differs between small and large luteal cells and with the age of the corpora lutea (Feng and Almond, 1998). Moreover, Yuan and Lucy (1996) reported that growth hormone and prolactin are stimulatory to progesterone secretion by large luteal cells, whereas small luteal cells are responsive to LH.

According to Lund et al. (1999), estradiol may play a key role in luteal function, which diminishes following ovulation induction of estradiol-deficient follicles.

3- Early embryonic development

Normal reproductive function leads to the production of healthy gametes, which are ready to be fertilized and will develop into conceptuses. Early embryonic development is a critical stage, and the events and factors that may interfere with the ability of the conceptuses to survive are the topics of the following section.

Fertilization occurs at the ampullary-isthmic junction of the fallopian tube. If oocytes fail to undergo complete maturation, abnormalities will result during fertilization and early development of the embryo (Leibfried-Rutledge et al., 1989). The two-cell stage lasts for 6 to 8 hours and the four-cell stage for 20 to 24 hours. Embryos are mainly at the four-cell stage when they enter the uterus, approximately 48 to 56 hours after ovulation (Ashworth, 1991). Embryonic RNA synthesis commences at the four-cell stage and this is believed to correspond to the transition from the maternal to the embryonic control of development, when proteins transcribed by the embryonic genome are synthesized (Jarrel et al., 1991). Embryos spend the following two to three days in the proximal portion of the uterine horns, before becoming more evenly distributed throughout the uterus.

The blastocyst stage in the pig is reached at day 5-6, usually when pig embryos comprise 16-32 cells. The embryo hatches from its glycoprotein covering, the zona pellucida, 6-7 days after mating. After day 12, embryos can no longer successfully move to a different site in the uterus. Conceptuses become regularly spaced within the uterus with no overlap of adjacent embryos. From day 11-12 pig blastocysts elongate rapidly from the 9-10 mm spherical stage to the 1000 mm long filamentous form by day 16 (Ashworth, 1991).

The second week of pregnancy is a particularly critical period for embryonic survival in pigs. Within that time, conceptus estrogen synthesis is initiated, spacing and final placement of conceptuses is completed, and the signal for extending the functional lifespan of the corpora lutea is received by the mother (Roberts et al., 1988). In the pig, maternal recognition of pregnancy begins about 11-12 days after the start of estrus (Geisert et al., 1991). Swine blastocysts signal their presence to the sow by synthesizing and releasing estrogens, and possibly other substances, that interact with the maternal system to allow pregnancy to continue (Pusateri et al., 1996a). Thus, maternal recognition of pregnancy can be defined as the method by which the conceptus prolongs the functional lifespan of the corpora lutea (CL) established after ovulation (Geisert et al., 1991). Cárdenas and Pope (1993) reported that delaying mating, relative to ovulation, decreased the size and estradiol secretion of blastocysts, but did not affect the extent of their morphological diversity. Furthermore, at least four viable embryos must be present at this stage, otherwise the CL will regress, resulting in the termination of pregnancy (Lambert et al., 1991).

The principal mechanism involved in the estrogenic component of the signal appears to be a decrease in uterine release of $PGF_{2\alpha}$. During the estrous cycle, the endometrium secretes $PGF_{2\alpha}$ in a pulsatile pattern into the uterine venous drainage (endocrine direction), causing luteolysis. In pregnancy, blastocyst estrogens interact with the endometrium to change the direction of endometrial $PGF_{2\alpha}$ secretion so that it is transported into the uterine lumen (exocrine secretion). As a result, $PGF_{2\alpha}$ concentrations in the blood leaving the uterus are decreased and luteolysis does not occur (Bazer and Thatcher, 1977; Pusateri et al., 1996b). In addition, Carnahan et al. (1996) demonstrated that oxytocin is the stimulus for $PGF_{2\alpha}$ secretion in pigs and that oxytocin-induced endometrial release of $PGF_{2\alpha}$ into the uterine vasculature is suppressed to maintain CL function during early pregnancy.

Plasma progesterone concentrations during early pregnancy can modify oviductal (Buhi et al., 1990) and endometrial (Roberts et al., 1993) development and secretory activities. Variability in progesterone synthesis may therefore lead to asynchrony between the embryo and uterus. Thus, the time and pattern of rise in plasma progesterone concentrations may be an important factor in determining the likelihood of an embryo remaining viable (Jindal et al., 1997).

Wiesak et al. (1992) have suggested that prostaglandins ($PGF_{2\alpha}$, PGE_2) exert both stimulatory and inhibitory effects on the activity of porcine luteal cells. They can also interact with LH and with each other, indicating multiple sites of action. Hence, the ratios of LH, $PGF_{2\alpha}$ and PGE_2 are important in the control of progesterone synthesis during early pregnancy in pigs (Wiesak et al., 1992).

3.1 Uterine secretions

The uterus plays a central role in the reproductive biology of mammals, as it is responsible for the production of an environment that can support the development of the embryo and fetus (Davis and Blair, 1993). The production of a suitable environment includes the synthesis and secretion of products, such as proteins (uteroferrin, retinol-binding protein, growth factors, plasmin/trypsin inihibitor and opioid peptides) and prostaglandins, by the uterine endometrium. Recently, the presence of folate binding proteins and folate receptors were characterized in the endometrium of swine (Vallet et al., 1999). Proteins secreted by the endometrium deliver nutrients to the developing conceptus. Uteroferrin contains iron and retinol-binding protein contains retinol, which are transferred to the fetal liver via the blood stream (Roberts and Bazer, 1988), and folate binding proteins deliver folic acid to the developing swine conceptus during early pregnancy (Vallet et al., 1999). Thus, factors that control endometrial secretion of these and other endometrial proteins likely influence the efficiency of conceptus development and uterine capacity (Vallet and Christenson, 1996).

Pope and coworkers (1990) proposed a model in which estradiol released into the uterine lumen, initially from the more developed blastocysts, advanced uterine development and secretions. On the other hand, the less developed blastocysts are considered to be more sensitive to uterine advancement than the more developed littermates. Consequently, the more developed blastocysts within a litter have a better chance of survival than the less developed ones. Thus, the asynchrony between uterus and conceptuses may be an important cause of embryo mortality (Pope, 1988). More recently, Vallet and Christenson (1996) reported that treatment of gilts with estrogens did not mimic the enhancement of endometrial protein secretion that occurs in the presence of the conceptus by day 60 of pregnancy in swine. Hence, other conceptus factors may be involved and could be important in controlling the flow of endometrial proteins, and therefore essential nutrients, to the developing conceptus during this period. Moreover, a single injection of estradiol inhibited the development of blastocysts that begin elongation within the next 12 h, but did not consistently alter development of blastocysts that grow to the large spherical stage during that time period (Cárdenas et al., 1997).

Trout et al. (1992) reported that estradiol stimulated secretion of endometrial retinol-binding proteins in the presence of progesterone. Indeed, in the recent study of Vallet et al. (1998), in which uterine protein secretion was determined in White crossbred and in Chinese Meishan gilts with and without progesterone treatment, it was demonstrated that the conceptus did not influence secretion of either total protein or retinol binding protein during pregnancy and that the onset of secretion of these uterine proteins may be controlled by progesterone. Moreover, estrogen secretion by the conceptus was advanced by early progesterone treatment, but increased uterine protein secretion preceded the increase in estrogen secretion. Intrauterine content of total protein, retinol binding protein, uteroferrin and estrogen, which were lower in Meishan compared to White crossbred gilts, may play a role in the increased fertility of the Meishan breed. According to Kaminski et al. (1997), the difference in estrogen secreted between the Chinese Meishan and Yorkshire conceptus is likely due to the expression of the steroidogenic enzymes cytochrome P450 17α –hydroxylase and cytochrome P450 aromatase, which are lower in the Meishan by day 11 of pregnancy.

3.2 Uterine capacity

The concept of uterine capacity in swine has been studied by different authors and methods (Bazer et al., 1969a; Webel and Dziuk, 1974; Knight et al., 1977; Christenson et al., 1987; Bennet and Leymaster, 1989). It has been suggested that limitation of uterine space available to fetuses (Rathnasabapathy et al., 1956), and competition among embryos for biochemical factors and nutrients (Bazer et al., 1969b), were important determinants of litter size. However, according to Dziuk (1968) there is little reason to suspect that crowding affects embryonic survival in gilts with less than 14 embryos. In contrast, the findings of Père et al. (1997) suggested that uterine capacity limited litter size even in gilts with normal ovulation rates, and most of the supernumerary fetuses died during the first month of gestation. Moreover, an ovulation rate greater than the number of pigs the gilt was able to keep alive until farrowing, resulted in lighter fetuses at term. The variation in litter size and (or) weight induce metabolic modifications of the gilt (greater mobilization of body lipids) due to the higher energy demand induced by larger and heavier litters.

Uterine crowding has been shown to have effects on fetal growth, which was probably due to small placental contact area (Knight et al., 1977), and according to Dziuk (1985), fetuses in the middle of the uterine horn are the most likely to have limited space and be smaller at birth than their littermates. Small placental contact area limits the uptake or utilization of nutrients by the fetus and (or) nutrient transfer across the placental membrane (Sterle et al., 1995). Indeed, the large fetuses are in the positions in the uterus corresponding to the largest placentae in early pregnancy (James, 1974).

Interestingly, Wilson and coworkers (1998) demonstrated that the Chinese Meishan pig exhibits an increased litter size by developing a smaller, more efficient placenta, taking up less space in the uterus than conceptuses of American and European breeds, while not decreasing fetal/piglet viability. The reduced placental size may allow more Meishan conceptuses to colonize the uterus compared to American and European breeds. Furthermore, placental size is largely determined by the uterus in which a conceptus is gestated until approximately day 90. After day 90, fetal breed-specific mechanisms maintain optimal fetal growth. Between day 90 and term, Meishan fetal growth depends on progressive increases in placental blood vessel density and requires no increase in placental size. In contrast, American and European breeds conceptuses seem to rely exclusively on placental growth to increase placental-endometrial surface area for nutrient exchange (Biensen, et al., 1998). Therefore, uterine type determines conceptus size and conceptus genotype controls placental efficiency (Biensen et al., 1999).

3.3- *In vitro culture of pig embryos*

Embryonic development to the blastocyst stage in the pig has been difficult to achieve under *in vitro* conditions. However, Reed et al. (1992) reported progress in showing that pig embryos could be cultured from the zygote to the blastocyst stage, overcoming what has been termed the "four-cell block" to *in vitro* development. Attempts to overcome this "*in vitro* developmental block" included culturing embryos in oviducts maintained in organ culture, coculture with oviductal or granulosa cells, culture in ligated sheep oviducts, supplementation with complex biological fluids such as serum or oviduct fluid, and various modifications of simple culture media (for review see Petters and Wells, 1993; Bavister, 1995). Although these achievements made early stages of embryonic development feasible *in vitro*, the adequate culture conditions for the preimplantation porcine embryo have yet to be determined.

Pig embryos can be developed *in vitro* from the zygote to the blastocyst stage in a simple culture medium containing BSA. However, further development and hatching of cultured pig blastocysts requires the presence of serum and amino acid supplementation of the culture medium (Koo et al., 1997). The source of BSA is important for the successful development of embryos in vitro (Long et al., 1999). According to Dobrinsky et al. (1996), BSA fraction V (BSA-V) reduced developmental competence of embryos, probably due to the presence of contaminants in the preparation of BSA-V that can be stimulatory or inhibitory of cell proliferation. On the other hand, BSA fatty acid-free preparations could have some or all contaminants removed by extraction procedures. In the recent study with bovine embryos, Krisher et al. (1999) reported that during the first three days of preimplantation development *in vitro*, there was little requirement for protein, but thereafter, protein stimulated development to the blastocyst stage. Moreover, embryos that grow in the presence of serum had elevated glycolytic rates and appeared dark and granular. Removing serum from the culture medium altogether and replacing it with BSA in only the final stages of culture, resulted in development equal to that with serum.

The supplementation of amino acids taurine or hypotaurine to culture medium improved embryo development in pigs (Peters and Reed, 1991; Long et al., 1999) and hamsters (Barnett and Bavister, 1992), while glutamine tended to decrease the rate of development of pig embryos in a dose-dependent way (Iwasaki et al., 1999). Furthermore, increased concentration of NaCl impaired development of embryos to the advanced morula/blastocyst stages (Beckmann and Day, 1993), although pig embryos were tolerant of a wide range of potassium concentrations when the osmolarity of the medium was maintained by adjusting the NaCl concentration (Kim and Menino Jr, 1997).

Both metabolism and development of embryos are altered by different culture media, implying a functional linkage between these two indicators of successful embryogenesis (Barnett and Bavister, 1996). Metabolic activity of an embryo may have predictive value for evaluating its viability. Glucose uptake and metabolism are related to embryo developmental competence, and probably reflect energy requirements of the embryo (Renard et al., 1980). These requirements include synthetic precursors for protein synthesis, ATP, and energy demands for the activity of the Na^+/K^+ ATPase necessary for expansion of the blastocoele cavity (Rieger et al., 1992).

Embryo development is strongly influenced by oocyte maturation conditions (Weston et al., 1996). Thus, the type of energy substrate or nutrient supplied during *in vitro* maturation of oocytes profoundly affects subsequent developmental competence (Rose-Hellekant et al., 1998). In addition, higher glycolytic rates in *in vitro* matured oocytes may reflect increased developmental competence, which is likely due to the greater availability of cellular energy (Krisher and Bavister, 1999).

According to Warner et al. (1998), preimplantation embryonic survival depends on two major morphological parameters: rate of development and degree of fragmentation. A fast rate of development and a low degree of fragmentation lead to a better chance of producing live offspring. Both rate of development and degree of fragmentation are genetically controlled, the former by the Ped (Preimplantation embryo development) gene, and the latter most likely by genes that mediate apoptosis. Moreover, it seems probable that regulation of apoptosis will prove to be a major mechanism that mediates both oocyte and preimplantation embryonic survival. In a subsequent study, McElhinny et al. (1998) demonstrated the expression of Qa-2 antigen (product of Ped gene) in both trophectoderm and inner cell mass of Ped fast (Qa-2+) mouse strains, which have significantly higher preimplantation embryo cleavage rates both in vivo and in vitro than Ped slow (Qa-2-) mice. Thus, the higher number of cells in the inner cell mass of Ped fast mice may explain the overall reproductive advantage that this strain has, since a critical number of inner cell mass are required for implantation and further embryonic development.

Interestingly, Youngs et al. (1993) observed that Meishan embryos developed more slowly and contained fewer blastomeres than Yorkshire embryos. Consistent with these findings, Rivera et al. (1996) demonstrated that the reduced number of cells present in Meishan embryos resulted from a selective reduction in the number of trophectoderm, but not inner cell mass, cells.

4- Mechanisms mediating nutrition and reproduction interactions

The interactions between nutrition and reproduction have been studied for many years, and several lines of evidence suggest that reproductive function may be impaired without adequate nutrition (Monget and Martin, 1997). However, the mechanisms by which nutrition specifically influences reproductive processes are not yet fully understood. In this section, the physiological mechanisms mediating nutrition-reproduction interactions are described to better understand this connection.

4.1 Historical studies that established the "Metabolic Theory"

Approximately 40 years ago, Kennedy and Mitra (1963), studying the food-restricted female rat, provided the first evidence relating energy and metabolism as a mechanism timing puberty. They suggested that a measure of somatic development could be acquired by the brain indirectly from alterations in energy balance associated with an increase in size. Therefore, food intake or the correlated metabolic rate may act as the normal signal to initiate puberty.

In 1970, Frisch and Revelle suggested that critical body weight and fat content were involved in triggering menarche in girls. They proposed that the attainment of a critical body weight resulted in characteristic change in metabolic rate that, in turn, modified the hypothalamic sensitivity to estrogen feedback. Furthermore, body fat might play a role in modifying estrogen metabolism in the female, contributing indirectly to the control of the critical steroid feedback system and hence triggering puberty. The nature of the metabolic "trigger" that relates growth to puberty was also discussed by Marshall and Tanner (1970) who emphasized the fact that a close correlation between a particular parameter of somatic development and the onset of puberty may only be symptomatic to the achievement of some "maturity state", which is located in the brain.

Later, Bronson (1986) demonstrated in prepubertal female rats a rapid recovery of LH pulses with realimentation and a full recovery of pubertal development with exogenous GnRH infusion, after a period of feed restriction.

Foster et al. (1989) suggested that the chronically undernourished, ovariectomized lamb provided a powerful experimental model for studying the connections between metabolism and reproductive neuro-endocrine function. Because its ability to produce LH pulses was highly dependent upon the level of nutrition, the lamb represented a sensitive system with which to explore metabolic modulation of neural mechanisms controlling LH secretion.

Additionally, the studies conducted by Cameron (1989) with gonad-intact adult male rhesus monkeys indicated that the suppression of reproductive axis activity started to occur very shortly after the initiation of dietary restriction via a suppression of GnRH neuronal activity. It was suggested that pituitary responsiveness to GnRH was not decreased during short-term fasting and provided strong evidence that the decrease in LH pulse frequency and testosterone during short-term fasting resulted from a decrease in the frequency of GnRH release.

Several studies conducted with pigs have demonstrated similar interactions between nutrition and reproduction. Armstrong and Britt (1987) reported the cessation of estrous cycles in gilts subjected to chronic feed restriction and that this effect on the ovary was caused at least in part by altering hypothalamic release of GnRH. Cox et al. (1987) demonstrated an enhancement of ovulation rate in gilts by increasing dietary energy and administering insulin during the follicular phase of the cycle. They suggested that insulin concentrations may alter GnRH frequency, as exogenous insulin or increasing energy intake significantly increased the frequency of LH release during the follicular phase. However, in subsequent studies, an increase in LH secretion was not necessarily associated with insulin-induced effects on ovulation rate (Cox et al., 1987), suggesting that local, gonadotropin-independent effects were involved. Consistent with these data, Flowers and coworkers (1989) reported that gilts provided additional dietary energy for at least 10 days prior to estrus ("flush feeding") exhibited greater ovulation rate and increases in plasma concentrations of FSH, insulin and episodic LH secretion compared to control gilts. Booth (1990a) reviewed similar studies in feed-restricted and refed prepubertal gilts. Beltranena et al. (1991b) also confirmed that increased plasma insulin may be an important metabolic cue, which in an independent or synergistic action with gonadotropins and IGF-I, mediated the ovulatory response to "flush feeding". Furthermore, "flush feeding" induced a fluctuation in the energy status of the animal in the absence of major changes in body weight or composition. Meurer et al. (1991) studied the role of insulin on reproductive function by examining the effects of diabetes mellitus during follicular growth in gilts. They observed that the ability of follicles to produce estradiol, testosterone and IGF-I was impaired in diabetic gilts, even though LH concentrations in serum were adequate. Further, direct evidence for a LH-independent effect of metabolic state on ovarian follicular development came from the study of Cosgrove et al. (1992), using progestagen treatment of prepubertal gilts during periods of realimentation. The extensive literature on gonadotropin-dependent and -independent mechanisms mediating effects of nutrition and metabolic state on ovarian function in the gilt and lactating sow have recently been reviewed by Prunier et al. (1993a), Prunier et al. (1993b), Quesnel and Prunier (1995), Booth et al. (1996), Cosgrove and Foxcroft (1996), Zak et al. (1997a) and Mao et al. (1999).

Taken together, all the studies mentioned above demonstrated that the attainment and maintenance of reproductive activity in different species depends on adequate levels of nutrient intake. The physiological mechanisms by which nutrition affects fertility will be discussed in the next section.

4.2 Physiological mechanisms mediating the interactions between nutrition and reproduction

4.2.1 Sites of metabolic effects on reproduction

Undernutrition may affect all levels of the hypothalamic-pituitary-ovarian axis, but the primary locus of altered function seems to be the forebrain, specifically at the level of forebrain GnRH-secreting neurons (I'Anson et al., 1991). A disruption in the hypothalamic GnRH pulse generator inhibits LH secretion. However, the resumption of normal feeding has been reported to restore LH pulsatility quickly (i.e. within minutes, or at the very most hours) in rats (Bronson, 1986), ewes (Foster et al., 1989), gilts (Booth et al., 1996), and male rhesus monkeys (Cameron and Nosbisch, 1991). However, the restoration of LH pulsatility occurred slowly after refeeding in energetically disrupted women (Loucks and Verdun, 1998). These authors suggested that this striking contrast between women and other mammals may be another clue to the unidentified mechanism mediating the effect of energy availability on LH pulsatility. According to Bringer et al. (1997), several candidates could be implicated as the "common link" in the modulation of the GnRH pulse generator by energy balance: a) alterations in metabolic fuels such as free fatty acids, amino acids and glucose; b) decrease in peripheral hormones such as insulin, IGF-I and leptin; c) impairment of neuronal signals; and d) chronic reduction of basal metabolic rate and thermogenesis which could affect hypothalamic secretion by changes in vascular flow and/or neurosecretion.

On the other hand, the effects of nutrition can also affect ovarian function directly, as seen in ovulation rate in gilts subjected to "flush feeding" (Flowers et al., 1989). In this situation, small to medium-sized, antral follicles are the main target tissue for the nutritional signals. There is evidence that members of the IGF system may be a component of those signals (for review see Monget and Martin, 1997). Furthermore, it has been demonstrated that deleterious effects of feed restriction on ovarian function may occur without any changes in the pattern of LH secretion in gilts (Cosgrove et al., 1992) and heifers (Mackey et al., 1999).

4.2.2 Availability of metabolic fuels

For many years, it was widely believed that undernutrition would impair reproductive hormone secretion only when a significant amount of body weight and/or body fat had been lost (Frisch, 1984). However, Bronson and Manning (1991) in their review argued against the notion that body weight or body fat plays a role in transmitting information about nutritional status to the reproductive axis. Probably, the most compelling finding against the role of body weight or body fat as a mediator is that short-term periods of fasting can rapidly lead to a suppression of gonadotropin secretion before any changes in body weight or body composition occur. Direct evidence for suppression of LH secretion independent of changes in body weight or composition came from the study of Cameron and Nosbisch (1991) in primates, where missing a single meal lead to a slowing of pulsatile LH and testosterone that was apparent within the first four hours after the meal was missed. Booth et al. (1994) also demonstrated in prepubertal gilts that changes in metabolic status can mediate short-term nutritional effects on reproductive function, in the absence of changes in age, body weight or composition. Furthermore, Cameron et al. (1991) reported a suppression in LH, FSH and testosterone secretion by only two days of fasting in men, and Olson and coworkers (1995) reported alterations in LH secretion during a 3-day fast in women. Taken together, these findings suggest that undernutrition may suppress the central neural drive to the reproductive axis, as pituitary responsiveness to GnRH remains normal (Rojdmark, 1987). Therefore, the mechanism by which undernutrition suppresses activity of the reproductive axis involves decreased circulating energy, rather than a change in body composition (Cameron, 1996).

Studies in several species have found that reproductive success is influenced by the general availability of metabolic fuels (e.g. glucose, fatty acids) (see Wade and Schneider, 1992 for review). In the study by Schneider and coworkers (1993), using metabolic blockers (2-deoxy-D-glucose, an inhibitor of glucose utilization; methyl palmoxirate, an inhibitor of fatty acid utilization), it was suggested that glucose might serve as a key metabolic regulator of estrous cyclicity in the adult female hamster. In addition, investigations in the sheep measuring LH during insulin-induced hypoglycemia raised the possibility that the decrease in LH secretion may occur through altering GnRH secretion (Clarke et al., 1990; Bulcholtz et al., 1996). Bulcholtz et al. (1996) demonstrated that during reduced glucose availability, the pituitary could readily release LH, and the GnRH neurosecretory system could be induced to release GnRH. Thus, a major influence of reduced glycolysis is to inhibit the system(s) controlling the frequency of GnRH secretion. Similar findings were also reported by Nagatani et al. (1996) in female and male rats, and moreover, the suppression of LH pulses was potentiated by gonadal steroids in both sexes, although female rats seemed to be more sensitive to the decrease in glucose availability than the males.

Murahasi and coworkers (1996) reported that glucose availability could influence LH secretion through a central sensor in the lower brain stem and that the area postrema might be an important glucosensor involved in the modulation of LH secretion. However, the area postrema may not mediate all information about the nutritional control of GnRH secretion, because metabolic signals other than glucose might play a role (Bucholtz et al., 1996). In this regard, Cagampang et al. (1992) reported that a mechanism involving the afferent vagal nerves, particularly the gastric vagus, has a major role in the suppression of pulsatile LH release during acute fasting in ovariectomized rats and that peripheral changes which occur during acute fasting are intimately related to GnRH secretory dysfunction in the brain. Furthermore, it should also be recognized that the overall availability of a metabolite such as glucose is dependent not only on circulating concentrations, but also on cellular uptake (Livingstone et al., 1995). The same authors reported that the availability of glucose to a cell could be high or low according to membrane transport regulation of the glucose molecule (insulin/glucagon ratio), and that this regulation was based upon the overall supply and demands for energy substrates in the developing individual. Therefore, the ability of glucose to influence GnRH secretion may depend upon the dynamic flux of glucose across cell membranes and its action within the cell as an oxidizable substrate or biosynthetic macromolecule rather than upon static peripheral glucose values.

In contrast to the above studies, Schreihofer and coworkers (1996) provided evidence that a change in circulating glucose levels is not a necessary component of the metabolic signal that stimulates LH secretion during refeeding in male rhesus monkeys. Thus, the mechanism that mediates the stimulation of LH secretion during refeeding is somehow dependent on the metabolic state of the body or the availability of metabolic fuels in general, rather than on blood levels of a particular substrate. Consistent with these results, Tokach et al. (1992a) also demonstrated in energy restricted, primiparous, lactating sows that glucose infusions did not result in an immediate increase in pulsatile LH secretion. In this case, however, the primary cause of inhibition on LH secretion may have been suckling-induced neuroendocrine reflexes (De Rensis et al., 1993; Quesnel and Prunier, 1995). Moreover, in studies conducted in ewes in which glucose was administered in the late luteal phase, no significant changes were observed in the pattern of LH secretion (Downing e al., 1995; Rubio et al., 1997). Conversely, short-term fluxes in glucose availability are important regulators of LH secretion in the prepubertal gilt (Booth, 1990). Therefore, a normal pattern of LH release can probably be sustained as long as the animal can maintain normal blood glucose concentrations (Downing et al., 1995).

In humans, growing evidence suggests that menstrual disturbances in athletes are related to the metabolic cost of high levels of energy expenditure without compensatory increases in dietary intake (for review, see Wade et al., 1996). Indeed, in short-term studies examining the acute independent effects of exercise stress and energy availability, Loucks et al. (1998) were able to prevent the disruption of LH pulsatility in exercising women by supplementing their dietary energy intake. Consistent with these findings, De Souza et al. (1998) also reported that energy deficiency was the factor that disrupted reproductive function in exercising women. Thus, according to Laughlin et al. (1998), nutritional factors may represent a common contributing factor to the development and maintenance of multiple neuroendocrine-metabolic aberrations underlying both psychogenic and exercise-related functional hypothalamic amenorrhea.

As illustrated above, there are differences among species, gender and reproductive status in the way animals respond to the availability of metabolic fuels. Therefore, the model used in such studies should be carefully evaluated.

4.2.3 Metabolic hormones

4.2.3.1 Insulin

There is evidence that insulin provides an important signal to the brain and to the hypothalamic structures in response to the changes in food intake and body composition (for review see Schwartz et al., 1992). Insulin could act in the central nervous system to modulate the activity of the hypothalamic neurons or at the pituitary level by increasing the sensitivity of gonadotropes to GnRH (Baskin et al., 1983). Moreover, insulin-binding sites are located in the median eminence, and insulin has direct stimulatory effects on the electrical activity of hypothalamic neurons in the rat (Schwartz et al., 1992) and indirectly can affect the availability of amino acid precursors of neurotransmitter synthesis in the brain (Fernstorm, 1983). Insulin administration during fasting has been shown to enhance the transport of tryptophan and further increase the brain content of serotonin, which affects LH secretion (Fernstorm, 1983). Indeed, insulin concentrations were reported to be low during nutritional deprivation in pigs (Cosgrove et al., 1992; Booth et al., 1994; Zak et al., 1997a; Mao et al., 1999), sheep (Miller et al., 1998), and primates (Williams et al., 1996).

As mentioned previously, insulin stimulates tissue uptake of glucose and fatty acids, promotes lipogenesis and glycogen synthesis and inhibits lipolysis (Wade and Schneider, 1992). Thus, insulin is important for directing the partitioning and utilization of ingested fuels (Schreihofer et al., 1996). Additionally, insulin has been implicated as necessary for reproductive activity in some studies with rodents (Siegel and Wade, 1979).

Studies conducted with pigs also provided evidence of the effects of insulin on reproduction. Cox et al. (1987) demonstrated that exogenous insulin and dietary energy acted synergistically to increase ovulation rate in gilts, and Flowers et al. (1989) and Beltranena et al. (1991b) also reported an increase in plasma concentrations of insulin in flushed gilts. In addition, greater ovulation rates were observed in sows by Clowes and coworkers (1994) when remating was delayed to the second post-weaning estrus. They reported that insulin concentrations were also increased by "skipping a cycle". The insulin increase indicated a return to a more anabolic state, which improves ovarian function.

In addition, effects of exogenous insulin administration were reported at the ovarian level by Matamoros et al. (1990) and Matamoros et al. (1991), as reducing atresia and increasing the number of potential ovulatory follicles. The role of insulin on ovarian function seems to involve enhancement of intrafollicular production of IGF-1 (Matamoros et al., 1991). In the study of Meurer and coworkers (1991) in diabetic gilts, a decrease in follicular steroids and IGF-1 and an increase in ovarian follicular atresia were reported.

It has been suggested that insulin might act to influence the GnRH pulse generator by influencing the hypothalamic availability of the amino acids substrates necessary for the synthesis of norepinephrine and serotonin, which are important neurotransmitters influencing the GnRH pulse generator (Bronson and Manning, 1991). Moreover, the findings of Downing and Scaramuzzi (1997) suggested that insulin could affect normal hypothalamo-pituitary ovarian feedback mechanisms in the cyclic ewe and they implicated insulin as a mediator of normal pituitary function.

In contrast to these findings, the study of Williams and coworkers (1996) in adult male rhesus monkeys, reported that pharmacological suppression of insulin secretion had no effect on meal-induced LH secretion, and therefore, insulin does not mediate the changes in reproductive hormone secretion that occur in response to changes in food intake. However, tissue fuel availability may play a key role in mediating nutrition-induced changes in the central neural drive to the reproductive axis.

4.2.3.2 Growth hormone (GH)/Insulin-like growth factor (IGF) axis

IGF-I is a peptide hormone involved in metabolic regulation of growth (Lamberson et al., 1996). Both GH and IGF-1 receptors exist on porcine granulosa cells and mediate effects by either endocrine (responses to circulating levels of GH or IGF-I) or autocrine/paracrine (local cellular or intercellular) pathways. Peripheral IGF-I concentrations are primarily controlled by the responsiveness of hepatic tissue to GH stimulation and insulin is a key regulator of hepatic sensitivity (Foxcroft, 1990).

Plasma concentrations of GH and IGF-I are responsive to plane of feeding and dietary composition (Booth, 1990b). During restricted feeding or when metabolic demands are high, low insulin status results in a decline in circulating IGF-I, even in the presence of high levels of GH (Booth, 1990a; Cosgrove et al., 1992; Charlton et al., 1993b). In this situation, GH exerts catabolic effects and ovarian activity is inhibited.

Additionally, fasting can decrease systemic IGF-I concentrations without affecting intraovarian IGF-I concentrations, suggesting that the production of ovarian IGF-I is differentially or more slowly regulated than that of the liver IGF-I production (Spicer et al., 1992). In this regard, Charlton et al. (1993b) observed that refeeding increased the expression of IGF-I mRNA in hepatic but not in ovarian tissue of prepubertal gilts. Thus, there is differential regulation of the IGF-I gene expression in porcine hepatic and ovarian tissues.

The study of Hiney et al. (1996), using small doses of IGF-I administered intraventricularly in the brain of prepubertal female rats, demonstrated that IGF-I of peripheral origin contributed to the initiation of female puberty by stimulating LHRH release from the hypothalamus, an effect that appeared to be amplified by the increased synthesis of IGF-I receptors in the median eminence during first proestrus. Furthermore, it was demonstrated that injections of IGF-I decreased the sensitivity of the hypothalamo-pituitary axis to estradiol negative feedback, and accelerated the process of puberty in the Rhesus monkey (Wilson, 1995). In the pituitary gland of the cow, the concentrations of IGFBP change in association with serum concentrations of progesterone during the estrous cycle (Funston et al., 1995). Taken together, these observations support a role for IGF-I in the control of GnRH neurosecretion in the hypothalamus and median eminence, and gonadotropin secretion by the pituitary gland. IGF-I should, therefore, be considered one of the major candidates for linking the metabolic status with reproductive function (see Monget and Martin, 1997 for review).

Additionally, Jenkins and Grossman (1993) suggested that IGFBP-1, either on its own or through its interaction with IGF-I, may also be a peripheral signal reflecting metabolic status and availability of fuel reserves. Such evidence was provided in the study of Laughlin and Yen (1996) with amenorrheic athletes, in which a marked increase in IGFBP-1 in these subjects, and its influence on IGF-I bioactivity, provided a peripheral signal of reduced metabolic fuel availability with a consequent slowing of hypothalamic GnRH pulsatility.

4.2.3.3 Thyroid hormones

Thyroid hormones are major regulators of energy homeostasis and the most notable effect is the increase in metabolic rate that follows their administration (Orban et al., 1998). Plasma levels of thyroxine (T4) and triiodothyronine (T3) are regulated by thyrotropin-releasing hormone (TRH), which is produced by neurons in the paraventricular nucleus, and regulates thyroid-stimulating hormone (TSH) secretion by the anterior pituitary (Orban et al., 1998).

Under normal conditions, only a small amount of T3 is secreted by the thyroid gland. The remaining T3, which is available for binding sites in the plasma and cells of the body, is generated in the peripheral tissues by monodeiodination of T4 by the enzyme 5'-deiodinase. This enzyme is found in peripheral tissues, such as kidney and liver, as well as in the brain. The activity of the liver and kidney enzymes are sensitive to the nutritional state of the organism and are found to be most active during states of accelerated glucose metabolism (characteristic of the fed state). Moreover, caloric restriction alters the concentrations of T3 and reverse T3 (rT3) by reducing the peripheral conversion of T4 to T3 and by reducing the metabolic clearance rate of rT3 and with minor associated changes in T4 (Danforth, 1983).

Serum concentrations of thyroid hormones represent, in the majority, hormone bound to circulating thyroid binding proteins. Indeed, level of nutrition induces changes in binding, with greater binding during over nutrition and reduced binding during undernutrition. However, free T3 is also increased with overfeeding and decreased with underfeeding, suggesting that changes in caloric intake induce similar changes in both total and free concentrations of T3 (Danforth, 1983).

Circulating concentrations of T3 vary directly with the amount of dietary energy needed to maintain energy balance and is affected by the carbohydrate content of the diet (Ulijaszek, 1996). According to Mariash and Oppenheimer (1985), the synergism between carbohydrate feeding and T3 was not due to an alteration in the production or turnover of T3, or to a change in the T3 nuclear receptor, and the precise mechanism remained to be defined. Furthermore, there is evidence that the role of thyroid hormone as a regulator of glucose metabolism is insulin-independent (Müller et al., 1989a; Müller et al., 1989b).

The role of thyroid hormones on reproduction has been studied in species such as sheep (Webster et al., 1991) and deer (Anderson and Barrell, 1998), whose seasonal pattern of reproduction is linked to photoperiodic signals involving the pineal hormone melatonin (Robinson et al., 1996). The study of Viguié et al. (1999) provided strong evidence that thyroid hormones can act directly within the brain, inhibiting GnRH/LH secretion, at the transition from the breeding season to anestrus. Besides these central effects, there is evidence that thyroid hormones can exert direct stimulatory effects on granulosa cell function, acting in synergism with FSH (Maruo et al., 1987). This interaction between thyroid hormones and gonadotropins regulates follicular steroidogenesis, depending on follicle size (Gregoraszczuk and Skalka, 1996). Consistent with these findings, thyroid hormone receptors have been reported in porcine (Wakim et al., 1987) and human (Wakim et al., 1993) granulosa cells.

4.2.3.4 Leptin

In the few years since leptin was identified as a satiety factor in rodents, it has been implicated in the regulation of various physiological processes, such as food intake, energy expenditure and whole-body energy balance in rodents and humans (see Houseknecht et al., 1998 for review).

The relatively recent discovery of leptin (Zhang et al., 1994) has generated considerable excitement in the area of reproductive biology. Many believe that this hormone could be the indicator of nutritional status that allows reproductive processes to proceed (Clarke and Henry, 1999). Direct evidence for this role of leptin came from the studies of Chehab et al. (1996) and Mounzih et al. (1997) where infertility of the genetically obese *ob/ob* mice (lacking endogenous leptin) was corrected by treatment with exogenous leptin in females and males, respectively. These studies also showed that the weight loss experienced by pair-fed *ob/ob* animals did not restore fertility, implying that obesity alone was not the cause of infertility in these animals. This strengthened the hypothesis that leptin was directly responsible for these changes in reproductive capacity. There is evidence that leptin plays a permissive role in the onset of puberty, probably through action on the hypothalamus, where leptin receptors are found in cells that express appetite-regulating peptides (Barb, 1999; Cunningham et al., 1999; Foster and Nagatani, 1999).

The mechanisms of leptin actions on reproduction remain unknown. However, leptin has been shown to stimulate GnRH secretion from the basal hypothalamus, suggesting that leptin may signal GnRH-containing neurons directly (Yu et al., 1997). Moreover, the discovery of leptin receptors on ovaries and testes (Cioffi et al., 1996), suggested that leptin may directly stimulate the gonads, promoting their function. In contrast to these findings, Zachow and Magoffin (1997) demonstrated that leptin could act directly on the ovarian granulosa cells to decrease estradiol but not progesterone production.

Furthermore, as circulating levels of leptin are directly related to body fat content, elevated levels of adipose tissue could have a positive influence on sexual responsiveness (Campfield et al., 1996).

4.2.4 Hypothalamic-Pituitary-Adrenal (HPA) axis

The effects of stress on reproductive function and the mechanisms mediating these effects depend on the type, duration and frequency of the stimulus (Rivier and Rivest, 1991). It is believed that the hormones of the HPA axis mediate the effects of stress on the reproductive system (Macfarland and Mann, 1977). Indeed corticotropin-releasing factor (CRF), pro-opiomelanocortin (POMC)-derived peptides (such as ACTH and β-endorphin), and adrenal corticosteroids play a very important role in mediating the effect of stress on reproductive function (Rivier and Rivest, 1991).

Stress-related hormones can influence sexual functions at all levels of the hypothalamic-pituitary-gonadal axis: the brain (to inhibit GnRH secretion), the pituitary (to interfere with GnRH-induced LH release), and the gonads (to alter the stimulatory effect of gonadotropins on sex steroid secretion) (Rivier and Rovest, 1991). In the study of Maeda et al. (1994), it was demonstrated that acute fasting induced stress-like responses, which activated the adrenergic input to the paraventricular nucleus and facilitated CRF release, thus leading to the suppression of LH secretion in female rats. In contrast to these findings, studies conducted in male rhesus monkeys (Schreihofer et al., 1993a; Schreihofer et al., 1993b) demonstrated that the extreme reduction in GnRH pulse generator activity due to missing a single daily meal, and its prompt reversal by refeeding or by intragastric infusions of an isocaloric nutrient solution, was the result of specific metabolic signals rather that the activation of the HPA axis. Consistent with these findings, studies conducted in sheep also indicated that the HPA axis was not involved in the decrease of LH secretion induced by hypoglycemia (Clarke et al., 1990; I'Anson et al., 1994).

There is, however, increasing evidence that the mechanisms mediating the effects of stress on reproductive function are primarily involved in the effects of prolonged but not acute stress stimuli (Rivier and Rivest, 1991). According to Liptrap (1993), the expected consequences of these effects in females may be disturbances of the estrous cycle, involving a high incidence of prolonged cycle length or poor follicular development, and a high incidence of failure in ovulation. Further evidence of the effects of stress on ovarian function came from the study of Montgomery et al. (1997) in pigs, in which it was suggested that *in vivo* exposure to elevated glucocorticoid concentrations, following ACTH treatment during the luteal phase of the cycle, influenced follicular steroid hormone production, which could subsequently compromise follicular development. These authors reported a reduction in IGF-I synthesis by granulosa cells, and impairment in IGF-stimulated progesterone production by granulosa cells, following ACTH treatment.

4.2.5 Endogenous opioids

Endogenous opioid peptides (EOP) are components of a family of neurotransmitters, which profoundly influence reproduction. It is suggested that the main site of opioidergic inhibition of LH is hypothalamic, via inhibition of GnRH and noradrenergic neurons (Barb et al., 1994). This inhibition requires the presence of gonadal steroids; thus, the withdrawal of opioid tone, probably due to a lowering in the concentration of opioid receptors, contributes to the pre-ovulatory LH surge (Jenkins and Grossman, 1993).

The EOP have also been suggested to have a vagally mediated role in feeding, as opioid receptors were associated with the vagal afferent system. Therefore, fasting-induced changes in the gastrointestinal tract, i.e. gastric constriction, gastric secretion or gastric motility, could activate an opioidergic mechanism, which has been reported to suppress LH release during acute fasting (Cagampang et al., 1992).

In pigs, there may be two opioidergic mechanisms modulating LH secretion. One is progesterone-dependent, as in the luteal phase, and the other is dependent on the suckling-stimulus during lactation (Cosgrove et al., 1993). In the cyclic gilt, the opioid antagonist naloxone was found to increase LH secretion only in the luteal phase, when the concentration of plasma progesterone was high (Barb et al., 1986). In the lactating sow, although suckling-induced suppression of LH secretion has been observed in the immediate postpartum period (De Rensis et al., 1993b; Sesti and Britt, 1994), in a previous study of De Rensis et al. (1993a), chronic naloxone treatment did not prevent this initial suckling-induced inhibition of LH secretion. In the recent study of De Rensis et al. (1998), it was demonstrated that the regulation of LH and prolactin secretion in lactating sows changed with time post partum. Thus, an opioid-dependent mechanism is an important component of the suckling-dependent regulation of LH and prolactin secretion in established lactation, but not during the first 72 hours postpartum (De Rensis et al., 1998). Furthermore, it was demonstrated that EOP also modulate LH and prolactin secretion during late gestation in the sow (Willis et al., 1996).

4.2.6 Sympathoadrenal system

The sympathoadrenal system consists of the sympathetic nervous system (SNS) and the adrenal medulla. The SNS releases noradrenaline and the adrenal medulla releases adrenaline (and a small proportion of noradrenaline) into the circulation. The activity of both systems is affected by dietary composition and plane of nutrition. In general, fasting suppresses SNS activity but stimulates the adrenal medulla, in contrast to overnutrition in which the opposite occurs (I'Anson et al., 1991).

Adrenaline and noradrenaline are known to directly affect GnRH release from the hypothalamus, and as their plasma concentrations increase during fasting, the sympathoadrenal system could provide a link between metabolic status and neurotransmitter pathways that affect the reproductive system (I'Anson et al., 1991). Furthermore, it was suggested that catecolamines stimulate both pulsatile and surge secretion of gonadotropins in swine (Estienne et al., 1997).

As mentioned previously, dietary peptides and amino acids are precursors to CNS neurotransmitters. In the study of Downing et al. (1997) using intravenous infusion of individual amino acids in ewes, no direct evidence was provided that increasing the supply of amino acids influenced ovulation rate or the secretion of gonadotropins. Therefore, an increase in the rate of synthesis of a particular neurotransmitter does not guarantee that this is coupled to a change in functional activity of the neurotransmitter.

4.2.7 Excitatory amino acids (EAAs)

Excitatory amino acid neurotransmission is an essential component of the neuroendocrine transmission line that regulates anterior pituitary LH and FSH secretion in pigs (Estienne et al., 1997). EAAs, such as glutamate and aspartate, are found in large concentrations in presynaptic sites of the hypothalamus, including the arcuate nucleus, the supraoptic nucleus, the paraventricular nucleus, and the preoptic area (Brann and Mahesh, 1994).

The action of EAAs is mediated by activation of different subtypes of postsynaptic receptors, which include N-methyl-D-aspartate (NMDA) receptors and kainate receptors, with the latter being activated specifically by kainic acid (KA) (Pinilla et al., 1998). Studies in female rats have demonstrated that systemic administration of NMDA or KA stimulates the secretion of LH in prepubertal and adult animals (Abbud and Smith, 1991; Carbone et al., 1992). Moreover, the study of Honaramooz et al. (1998) provided further evidence of the role that EAAs, through NMDA receptors, play in prepubertal development of LH and FSH secretion in heifer calves; their effects are age related, developing in the early postnatal period and reaching their maximum in the mid to late prepubertal period, sometime prior to the first ovulation.

The study of Sesti and Britt (1991), using the excitatory amino acid NMDA, demonstrated in sows that suckling acts to block the release of GnRH at the hypothalamic level. More recently, Popwell et al. (1996) demonstrated that EAAs both inhibited and stimulated LH secretion in prepubertal gilts and barrows, depending on the site of action within the brain-pituitary unit.

4.2.8 Neuropeptide Y (NPY)

NPY has been implicated in the hypothalamic regulation of reproduction and energy homeostasis (Kalra and Kalra, 1996). In addition to its effects on feed intake, NPY may modulate the secretion of GnRH from the hypothalamus, and LH release from the pituitary gland (Wade et al., 1996).

In mice, there is evidence that acceleration of episodic GnRH discharge necessary for the preovulatory LH surge is also dependent upon an increase in hypothalamic NPY output (Kalra and Crowley, 1992). Additionally, NPY may contribute to maintain LH secretion when GnRH secretion is either dimished or markedly suppressed in response to environmental challenges such as nutritional deficiency or undernourishment (Bronson, 1989; Kalra and Kalra, 1996). As NPY concentrations are enhanced under these conditions, Kalra and Kalra (1996) proposed that the increased amount of NPY reaching the pituitary may compensate for the loss of GnRH, and thereby, sustain LH secretion, although at a lower range. In contrast to these findings in mice, it has been demonstrated that ICV administration of NPY suppresses LH secretion in pigs (Barb, 1999) and ewes (McShane et al., 1992).

Overall, this review showed different mechanisms and different effects (central, local) of nutrition manipulation on reproductive function in several species (rodents, sheep, cattle and pigs). Moreover, it was shown that nutrition may also impact early embryonic development, which in turn may contribute to increase embryo mortality. In the following chapters, a series of experiments describe the effects of nutrition manipulation during the estrous cycle on subsequent fertility in gilts, and the possible mechanisms that are mediating these effects.

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Figure 2.1 Metabolic pathways for glucose-6-phosphate in the liver (Redrawn from Lehninger, 1993).

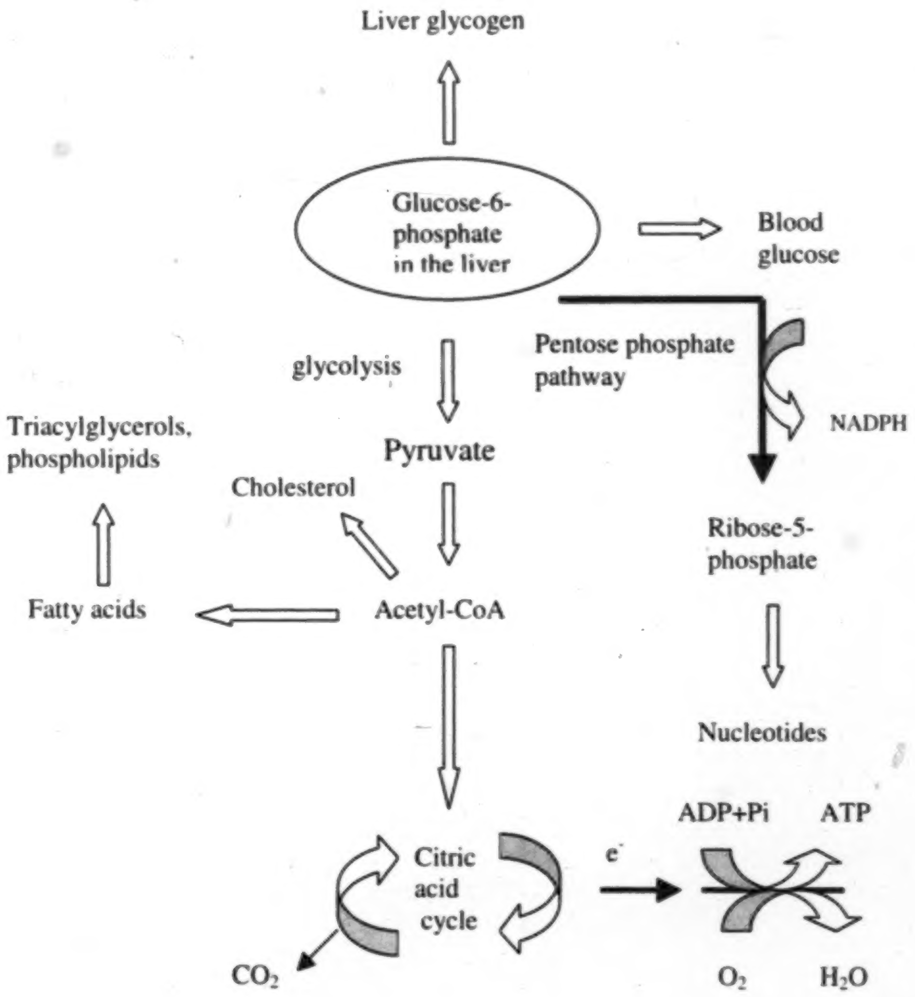

Liver glycogen
Glucose-6-phosphate in the liver
Blood glucose
glycolysis
Pentose phosphate pathway
NADPH
Triacylglycerols, phospholipids
Pyruvate
Cholesterol
Ribose-5-phosphate
Fatty acids
Acetyl-CoA
Nucleotides
ADP+Pi
ATP
Citric acid cycle
e⁻
CO₂
O₂
H₂O

Figure 2.2 Metabolism of fatty acids in the liver (Redrawn from Lehninger, 1993.

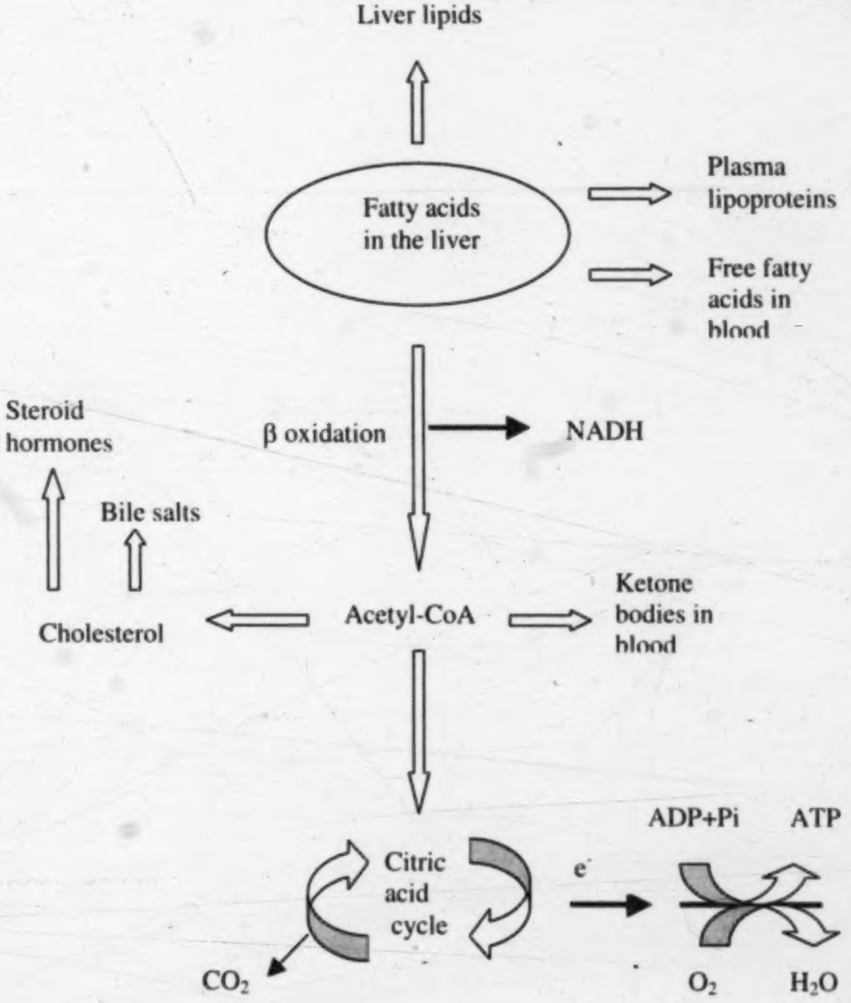
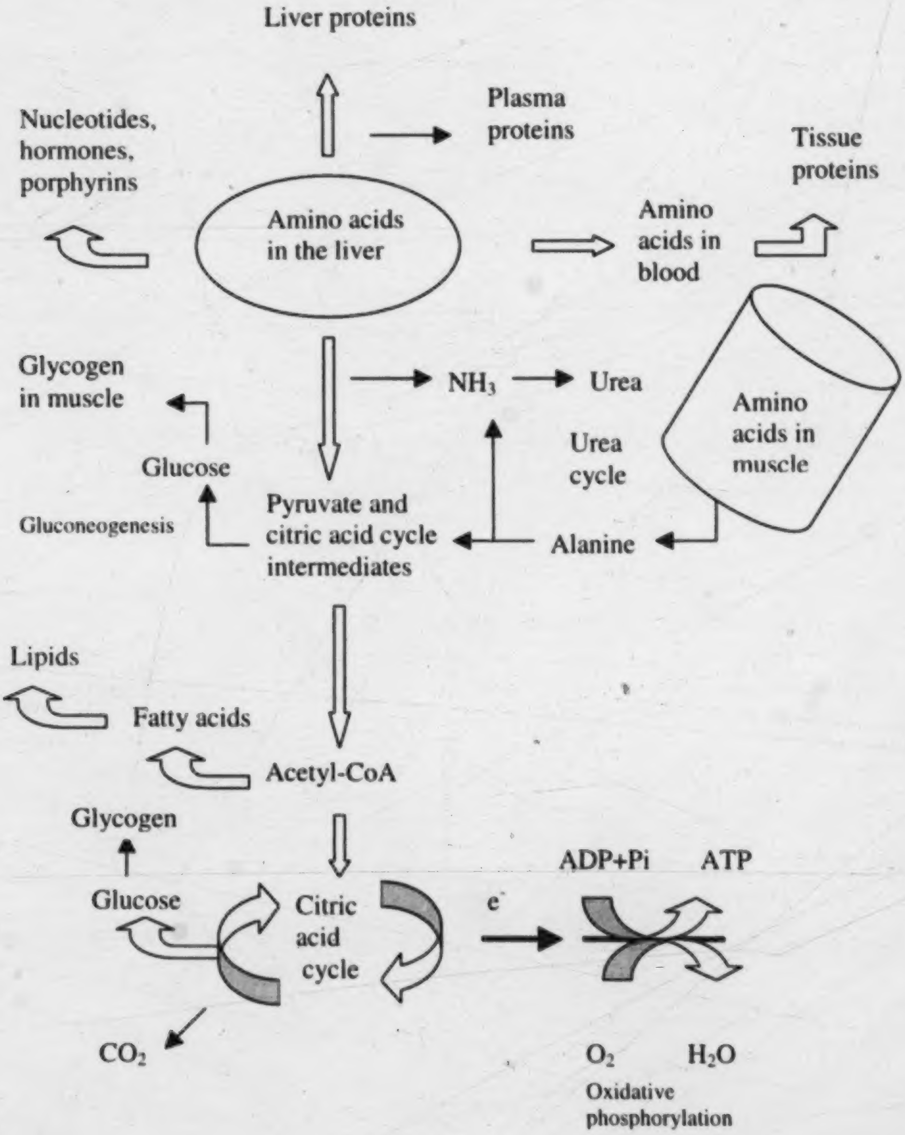

Liver lipids
Fatty acids in the liver
Plasma lipoproteins
Free fatty acids in blood
Steroid hormones
β oxidation
NADH
Bile salts
Ketone bodies in blood
Cholesterol
Acetyl-CoA
ADP+Pi
ATP
Citric acid cycle
e⁻
CO₂
O₂
H₂O

Figure 2.3 Metabolism of amino acids in the liver (Redrawn from Lehninger, 1993).


Liver proteins
Plasma proteins
Nucleotides, hormones, porphyrins
Tissue proteins
Amino acids in the liver
Amino acids in blood
Glycogen in muscle
NH$_3$
Urea
Amino acids in muscle
Urea cycle
Glucose
Gluconeogenesis
Pyruvate and citric acid cycle intermediates
Alanine
Lipids
Fatty acids
Acetyl-CoA
Glycogen
ADP+Pi
ATP
Glucose
Citric acid cycle
e$^-$
CO$_2$
O$_2$
H$_2$O
Oxidative phosphorylation

CHAPTER 3

CONSEQUENCES OF DIFFERENT PATTERNS OF FEED INTAKE DURING THE ESTROUS CYCLE IN GILTS ON SUBSEQUENT FERTILITY

3.1 Introduction

Reproductive efficiency is an important goal in the swine industry and is represented by the number of pigs weaned per sow per year (Foxcroft et al., 1995). Replacement gilts are a critical component of the breeding herd and it is essential to improve fertility in these animals, emphasizing genetics, nutrition, and management practices.

Several studies have demonstrated the interactions between nutrition and reproduction in gilts (Armstrong and Britt, 1987; Cox et al., 1987; Booth et al., 1994) and lactating sows (Koketsu et al., 1994; Zak et al., 1997a). In cyclic gilts, a positive relationship exists between feed intake and ovulation rate (Flowers et al., 1989; Beltranena et al., 1991) and nutritional effects on ovulation rate appear to be insulin dependent (see Cox, 1997). Even short periods of feed restriction in the prepubertal gilt produce inhibitory effects on ovarian development (Cosgrove et al., 1992; Booth et al., 1994). However, the effects of different patterns of feeding during the 19 d period of pre-ovulatory follicular development predicted by Morbeck et al. (1992) on subsequent fertility are still unclear.

In recent studies in primiparous sows (Zak et al., 1997a), nutritional changes were used to create different patterns of catabolism during a lactation period of 28 d and differentially affected reproductive performance. Low ovulation rates and marginally extended weaning-to-estrus intervals were observed in response to any pattern of feed restriction compared to sows fed to appetite throughout lactation. However, decreased embryo survival to d 28 was only observed in sows subjected to restriction in late lactation.

The aim of the present experiment was to develop a comparable gilt model to further test the hypothesis that different metabolic states, achieved by different patterns of feed restriction at critical times before recruitment of follicles into the pre-ovulatory hierarchy, will differentially affect the reproductive performance of gilts.

3.2 Materials and Methods

3.2.1 Pre-treatment of gilts

The first target of the study was to have groups of growth-matched, littermate gilts with an age of 160 d and around 90 kg body weight when first stimulated with vasectomized boars. To achieve this target, prospective littermates (Pig Improvement Canada Ltd, Camborough x Canabrid terminal line) were identified at weaning and were fed a series of three diets ad libitum; a weaner diet until approximately 25 kg body weight, a grower diet until around 60 kg body weight, and finally a gestation, rather than a finisher, diet to limit their growth rate until about 90 kg body weight (Table 3.1). Throughout this period, gilts that had very high or very low body weights compared to their littermates were taken out of the study to create littermate groups with similar prepubertal growth rates. Any lame gilts were also culled from the experiment.

Gilts which remained in the study were directly exposed to vasectomized boars for at least 15 min daily from 160 d of age (6 gilts: 1 boar) until the last gilt had displayed pubertal estrus (data on body weight and backfat at 160 days of age and age, body weight and backfat at pubertal estrus are presented in Appendix 3.1 and 3.2. At this stage, gilts were weighed, backfat thickness at P_2 was measured (Renco Lean-Meter, Renco Corporation, Minneapolis, MN, USA) and individually fed a grower diet twice daily at a level to meet their metabolic requirements, and to achieve a growth rate of 750 g/d, which was considered to be achievable using the diet fed, the genetic potential of the gilts, and expected voluntary feed intake.

Digestible energy (DE) requirements for maintenance were calculated as metabolic body weight ($BW^{.75}$) X 110 kcal (NRC, 1998), and the energy requirements for growth were estimated as 4 Mcal, based on the assumption that tissue accretion comprises 77 % lean and 22 % fat (C. de Lange, 1997, personal communication) and that the amount of heat produced by 1 g of lean is 1.25 Mcal and 1 g of fat is 9.5 Mcal, (9.5 x .22 = 2.1 Mcal for fat deposition per day, plus 1.25 x .77 = 0.96 Mcal for lean deposition per day, giving a total energy requirement for growth of 2.1 + .96 = 3.06 Mcal). As digestible energy is used for tissue deposition with an efficiency of 77% (Noblet et al., 1990), the total DE requirement for growth was estimated as 3.06/77 = 3.94 or 4 Mcal.

On the basis of these calculations, the energy requirement for maintenance, plus 4 Mcal, divided by the energy content of the diet (grower diet = 3314 kcal DE), gave the amount of feed intake per day. Gilts were weighed weekly (without restrictions of feed or water), and their feed intakes were adjusted within a 10 kg range of body weight, equivalent to feed increments of 100 g (Appendix 3.3). Data on weekly growth rate of gilts are presented in Appendix 3.1.

To synchronize second estrus of the females within a littermate group, from d 15 of their first estrous cycle, each gilt received the oral progestagen altrenogest (Regu-Mate, Hoechst-Roussel Vet., Canada) until the last littermate in heat received at least 5 days of Regu-Mate treatment. After the withdrawal of Regu-Mate, gilts were fed to appetite twice daily (0700 and 1400 h), and daily feed weigh backs were carried out to estimate "unrestricted" feed intake at this stage. Gilts were tested for estrus twice a day (0700 and 1900 h) with mature vasectomized boars until the onset of second heat. All gilts came in heat within 6 days after Regu-Mate withdrawal with a mean interval of 26 days between first and second estrus. Infertile breedings were allowed at second estrus as the best way to confirm standing heat, and to maintain good libido in the vasectomized boars.

3.2.2 Experimental treatments

All experimental procedures were carried out in accordance with the guidelines of the Canadian Council for Animal Care and under authorization from the Faculty Animal Policy and Welfare Committee of the University of Alberta.

After the withdrawal of Regu-Mate, littermate gilts were randomly allocated to one of three feeding regimens, HH (a positive control group), HR and RH. If it was not possible to allocate three littermates, pairs of gilts were randomly allocated to the two main treatments (HR, RH) and matched with a non-littermate HH gilt according to body weight and age at puberty. Of a total of 23 groups, seven were matched with a non-littermate gilt.

To match the duration of feed restriction to the same 7-d periods used in previous lactating sow work (Zak et al., 1997a), but still allow a period of unrestricted feeding in all treatment groups during the final phase of follicular growth (equivalent to the weaning-to-estrus period in the sow), different patterns of feed allowance were imposed between d 1 to 7 (early luteal phase) and d 8 to 15 (late luteal phase) of the cycle; all gilts were then fed at the same high rate of intake between d 16 and estrus (follicular phase). All feed allowances were equally divided between two meals fed at 0700 and 1400 h.

Defining d 0 as the first day of the second standing heat, the HH, HR and RH treatments were applied as follows:

Group HH - Energy feed intake was set at 95 % of "unrestricted" energy intakes established during the period of feeding from d 16 and onset of second standing estrus. This was equivalent to an energy intake of 2.8 X maintenance and was fed to HH gilts from d 1 to 7 and d 8 to 15 of the cycle based on metabolic body weights at d 0 and d 7, respectively. This energy allowance ensured that 1) all gilts would consume their allotted feed, and 2) that the restriction in energy intake relative to "unrestricted" feeding was proportional to the energy deficit seen in the unrestricted first parity sows in late lactation in the study of Zak et al. (1997a).

Group RH - From d 1 to 7 the energy allowance of these gilts was set 25% below that of the HH group, i.e. 2.1 X maintenance. This level of energy restriction was considered to be proportionally the same as that imposed in sows restricted during the last week of lactation, compared to unrestricted sows in the study of Zak et al. (1997a). From d 8 to 15, RH gilts were fed as HH gilts at 2.8 X maintenance based on metabolic body weights at d 7.

Group HR - Was fed the same as HH gilts from d 1 to 7, and then at 2.1 X maintenance from d 8 to 15 based on metabolic body weights at d 7.

From d 16 until breeding during their third standing estrus, all gilts received 2.8 X maintenance allowances based on d 15 metabolic body weights. Backfat was measured in all animals at d 0, d 7, d 15 and at the onset of estrus. Gilts were checked for estrus using the back pressure test during periods of fence line contact with mature boars twice daily (0700 and 1900) and were artificially inseminated 12 and 24 h after the first observed standing estrus with pooled semen (3 x 10^9 spermatozoa/dose) from the same group of boars (Alberta Swine Genetics Corporation, Leduc, AB, Canada) specifically designated for this experiment. Immediately after first mating, all gilts received 1.5 X maintenance requirements (2.05 ± 0.11 kg) until slaughter at d 28 of pregnancy. The diets were nutritionally balanced in terms of amino acids, vitamins and minerals to meet NRC (1988) recommended nutritional requirements.

All gilts were slaughtered at a local abattoir on d 28 ± 3 of pregnancy. Reproductive tracts were recovered and ovulation rate was estimated by counting the number of corpora lutea on each ovary. To collect data on placental and embryonic development, the wall of the uterine horns was cut longitudinally along the antimesometrial side, starting at the utero-cervical junction. Before removing any embryos from the uterus, allantoic fluid volume was determined by draining this fluid into measuring cylinders. The length and width of the allantochorionic placenta, excluding the necrotic tips of the chorion, were measured *in utero* under minimal stretch and used to calculate placental area. The number of live embryos and crown-rump length (size) of each embryo were recorded. To provide an objective measure of abnormal development, embryos were classified as being non-viable on the basis of a crown-rump length two standard deviations less than the mean for all embryos recovered from that gilt (Jindal et al., 1996). Embryo survival was expressed as the percentage of corpora lutea represented by live embryos recovered. To determine whether the previous feeding regimen had subsequent effects on the pattern of circulating progesterone in early pregnancy, a series of blood samples were taken by acute venepuncture 36, 48, 72, and 96 h after the onset of estrus and plasma progesterone concentrations determined using an established radioimmunoassay (Coat-a-Count Progesterone, Diagnostic Products Corporation, Los Angeles, CA, USA) previously validated for use with porcine plasma without extraction (Mao and Foxcroft, 1998). The sensitivity of the assay, defined as 88% of total binding, was 0.01 ng/tube. The intra- and interassay CV were 8.0 and 18.5%, respectively.

3.2.3 Statistical analysis

The data were analyzed as a randomized complete block design, with three treatments in 23 blocks, each block consisting of a group of three littermates. Treatment effects on ovulation rate, embryonic survival, body weight and backfat changes to estrus onset were analyzed using the general linear model (GLM) procedure of the Statistical Analysis System (SAS, 1990). Embryonic survival data were arcsine transformed before analysis. The analysis of body weight and backfat changes to estrus onset included the effects of block and treatment in the model with body weight and backfat at d 0 as covariates. Since it was not possible to slaughter all gilts exactly at d 28 of pregnancy, correlation analyses were performed using residuals of embryo size, placental area, allantoic fluid volume, and embryo number. All these embryo parameters were based on averages per gilt and were corrected for block, treatment, day, and day by treatment interaction. As progesterone concentrations did not present a normal distribution, the data were log transformed prior to analysis and were analyzed by repeated measures analysis of variance, using the repeated measures general linear model (GLM) procedure of SAS (1990). The complete model included treatment, block and time as the main effects, gilt was the experimental unit, and gilt within treatment by block interaction was used as the error term. In the event that significant treatment effects were established, multiple comparisons were performed using probability of differences (pdiff) between least square means, adjusted by Tukey-Kramer (SAS, 1990).

3.3 Results

3.3.1 Animals

Of the 69 gilts initially allocated to treatment, eight were excluded from the experiment. In the HH group, one gilt had a prolonged estrous cycle (> 24 days). In the HR group, one gilt had a polycystic ovary at slaughter, and three other gilts did not present a well defined standing heat, and were not pregnant at d 28. In the RH group, one gilt had a broken leg and two others had prolonged estrous cycles.

3.3.2 Body weight and backfat changes

Feed intake, body weight and weight change, backfat thickness at P_2 and backfat change of gilts are summarized in Table 3.2.

RH gilts had a lower growth rate ($P = 0.0056$) during the period of feed restriction (d 1 to d 7) compared to both HR and HH groups. The HR group also had a lower growth rate ($P = 0.0001$) during the period of feed restriction (d 8 to d 15) compared to their HH and RH counterparts. There was no difference ($P > 0.05$), in growth rate among groups from d 16 until onset of estrus probably due to their low feed intake in this period. Backfat changes were not different among groups during the experimental period. However, backfat measurements were greater in HH gilts on d 15 ($P = 0.0445$) and at estrus ($P = 0.02$) compared to RH gilts, with backfat in HR gilts being intermediate between the other two treatments.

3.3.3 Embryonic survival rate

Although ovulation rate, and number and size of embryos in utero per se, were not affected by treatment, embryonic survival was lower ($P = 0.038$) in HR gilts compared with HH and RH gilts (Table 3.3). Interestingly, a significant litter effect was observed for ovulation rate among gilts ($P = 0.03$).

3.3.4 Progesterone concentrations

Plasma concentrations of progesterone in early pregnancy were affected by previous nutritional treatment ($P < 0.05$). Progesterone concentrations in all treatment groups increased with time (Figure 3.1). However, this increase occurred later in HR compared with HH and RH gilts, with a difference in plasma concentrations evident at 48 (0.8 ± 0.2 vs 1.4 ± 0.2 and 1.2 ± 0.2 ng/mL in HH and RH, respectively; $P = 0.009$) and 72 h (3.6 ± 0.5 vs 4.9 ± 0.4 and 5 ± 0.5 ng/mL in HH and RH, respectively; $P = 0.049$).

3.3.5 Embryo-placental associations

Placental area and volume were not affected by previous nutritional treatments ($P > 0.05$) but were affected by litter of origin ($P = 0.009$ and $P = 0.003$, respectively).

Notwithstanding this litter effect, allantoic fluid volume and area of the allantochorionic membranes were correlated (Volume = 0.0073 x (area) + 37.6, $r = 0.35$, $P = 0.005$). Placental area was also positively correlated to embryo size at d 28 (Embryo size = 0.0003 x (area) + 18.35, $r = 0.28$, $P = 0.03$; Figure 3.2), and placental volume was negatively correlated to the number of embryos in utero (Placental volume = -4.317 x (number) + 207.55, $r = -0.39$, $P = 0.002$; Figure 3.3).

3.4 Discussion

Food availability is certainly the most important environmental factor that can affect growth. This was suggested by Williams et al. (1974) in a study in which rats were allowed to grow normally until a particular stage of development, and further growth was then inhibited by restricting the amount of food available. Bikker et al. (1996) also demonstrated in finishing gilts that realimentation after a period of feed restriction resulted in compensatory gain and increased feed efficiency. Furthermore, Booth et al. (1994) reported that gut fill clearly played a role in live weight variation and made a greater contribution to live weight in gilts fed to appetite. Therefore, the lower growth rate of HR and RH gilts when subjected to feed restriction and a trend towards compensatory growth after realimentation was expected. The lack of an effect on backfat change is likely due to the modest level of feed restriction used (2.1 X maintenance) which allowed the animals to continue growing, even though at a slower rate than their high plane fed counterparts. However, HH gilts had greater backfat measurements on d 15 and at estrus than RH gilts.

Treatment affected embryonic survival rate at d 28 of pregnancy, without affecting ovulation rate. It has been established that the recruitment of preovulatory follicles occurs between days 14 and 16 of the porcine estrous cycle and coincides with luteolysis of the corpora lutea (Hunter and Wiesak, 1990). In addition, because the size of the proliferating pool of follicles may be an important determinant of ovulation rate, factors that affect the size of this pool are of practical significance: genotype and nutrition have been clearly implicated (Dailey et al., 1972; Clark et al., 1973; Dailey et al., 1975; Hunter et al., 1993). Other studies have also demonstrated effects of nutrition on ovulation rate. Cox et al. (1987) demonstrated an enhancement of ovulation rate in gilts by increasing dietary energy and administering insulin during the follicular phase. Consistent with these data, Flowers and coworkers (1989) reported that gilts provided additional dietary energy for at least 10 days prior to estrus ("flush feeding") exhibited greater ovulation rate. Furthermore, short-term changes in feed intake induce fluctuations in the energy status of the animal in the absence of major changes in body weight or composition (Booth, 1990).

Feeding the high plane of nutrition to the feed restricted groups from d 16 of the cycle in the present study presumably allowed the gilts to recover from their less anabolic state, such that follicular recruitment into the preovulatory pool, and hence ovulation rate, were not affected by treatment. Furthermore, the level of feed restriction imposed was not too severe (2.1 X maintenance), and even if continued to ovulation, may not have markedly affected ovulation rate among groups.

Although ovulation rate was not affected, results from previous studies in lactating and weaned sows (Zak et al., 1997a,b), in which the restricted animals had an energy deficit of 25% compared with the counterparts fed ad libitum, led us to hypothesize that a relative decrease in metabolic state late in the estrous cycle may still have a detrimental effect on subsequent fertility by affecting the status (maturation) of the ovarian follicles available for recruitment into the ovulatory population. Nutritionally dependent changes in intra-ovarian regulators of follicular function could, therefore, be a possible explanation for the low embryonic survival in the HR group, which was restricted during d 8 to 15 of the estrous cycle. Consistent with the studies in lactating and weaned sows (Zak et al., 1997a,b) and on the relationship between follicle status and oocyte maturation in the pig (Ding and Foxcroft, 1994), metabolic responses to feed restriction at critical stages of follicular growth in the estrous cycle may affect oocyte maturation and subsequent embryonic development. Direct evidence to support this possibility is being obtained using an extension of the experimental model used in the present study.

Furthermore, gilts which had the lowest embryonic survival rate at d 28 of pregnancy also had the lowest progesterone concentrations in early pregnancy (48 and 72 h after onset of estrus). Studies involving manipulation of feed intake around the time of estrus in gilts (Ashworth, 1991; Pharazyn et al.,1991; Jindal et al. 1996), or during lactation in sows (Baidoo et al. 1992; Zak et al., 1997a), also established differences in embryonic survival. Moreover, Ashworth (1991) and Jindal et al. (1997) demonstrated in gilts that progesterone injections in the early stages of pregnancy could counteract a nutritionally induced increase in embryonic loss. Thus, periovulatory progesterone could be the mediator of nutritionally induced effects on embryonic survival in gilts.

Studies in ewes have also suggested that the level of priming progesterone, modulated by pre-ovulatory nutrition, influenced embryo survival through direct effects on the developing oocyte (McEvoy et al., 1995a). In subsequent studies, McEvoy et al. (1995b) reported that the provision of supplementary progesterone to ewes on a high plane of feeding during the pre-ovulatory priming phase elevated plasma progesterone levels and enhanced subsequent ovum development.

This raises the additional possibility, previously discussed by Hunter and Wiesak (1990), that differences in follicular maturation before ovulation may be reflected in subtle but physiologically important differences in progesterone secretion in early pregnancy. The transient nature of the treatment-induced differences in plasma progesterone (48 and 72 h after onset of estrus) again confirms the importance of monitoring the patterns of progesterone secretion at critical times in early pregnancy (Foxcroft, 1997).

Variability in progesterone synthesis may lead to asynchrony between the embryo and uterus and the time and pattern of the rise in plasma progesterone concentrations may be an important factor in determining the likelihood of an embryo remaining viable (Ashworth et al., 1989; Jindal et al., 1997). Therefore, the slower rise in progesterone levels in the HR gilts may be a cause of the lowest embryonic survival rate in this group.

As reported previously (Deligeorgis et al., 1984; Deligeorgis et al., 1985), our results showed that there were marked litter effects for some of the parameters analyzed, including age, body weight and backfat at first estrus (data not shown), ovulation rate and placental area and volume. Hence, the use of littermates is highly recommended in such experiments to eliminate the effect of family and avoid biased results.

There is evidence that the development of day 28 conceptuses (embryos and their extra-embryonic membranes) is related to the extent of embryonic loss during the first four weeks of pregnancy (Lutter et al., 1981). Hence, factors that are associated with embryonic mortality might also be associated with embryonic development. The uterus and its secretions mediate such effects, since embryos depend on uterine secretions for their development and they can only survive if the uterine environment develops in synchrony with their own development (Roberts and Bazer, 1988). However, the present results indicate that placental and embryonic size in the HR gilts with a higher embryonic mortality (32%) were not different from the groups with lower embryonic mortality (17%). Lack of placental development did not, therefore, appear to mediate nutritionally induced effects on embryonic survival to d 28 in this experimental paradigm. Indeed, placental volume was negatively correlated to the number of surviving conceptuses, suggesting that when larger numbers of embryos survive the implantation process, uterine capacity may already be limiting placental growth. This is consistent with evidence from Biensen et al. (1998) that the uterine environment has a dominant effect on placental development up to d 90 of gestation. The data in Table 3.4 support previous evidence (see Foxcroft, 1997) that post-implantation loss of conceptuses may be the major component of pre-natal loss in modern swine genotypes. The total number of pigs born to comparable gilts in our research herd is close to 10 (G. Foxcroft, personal communication), indicating that of a total pre-natal loss of up to 45% of ova ovulated, only 17% occurs in the pre-implantation period compared to 28% loss after d 28. The observed negative association between embryo numbers at d 28 and placental volume suggests that placental insufficiency may ultimately lead to subsequent loss of embryos in later gestation. If high embryonic survival rates to d 28 of gestation are typical of modern genotypes, and this is reflected in a trend towards smaller placental size in early pregnancy, the implications for both fetal birth weight and post-natal growth capacity merit careful evaluation.

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Table 3.1 Dietary compositions as formulated of weaner, grower and gestation rations used in the experiment

Ingredients %	Weaner	Grower	Gestation
Wheat	26.8	-	-
Barley	9.8	75.9	86.0
Soybean meal	15.8	12.0	4.0
Canola meal	-	6.1	4.5
Oat groat	19.5	-	-
Fish meal	4.2	-	-
Lysine	0 .2	0 .2	-
Whey	15.6	-	-
Limestone	1.0	1.1	1.6
Dicalcium phosphate	1.2	0.9	1.4
Salt	0.5	0.4	0.5
Oil	4.3	2.3	1.0
Premix [a]	1.1	1.1	1.0
Chemical analysis			
Digestible energy, kcal/kg	3,544	3,314	3,120
Crude protein %	20.15	17.30	13.75
Calcium %	0.86	0.72	0.93
Total phosphorus %	0.75	0.66	0.70
Lysine %	1.30	0.99	0.56
Methionine + Cystine, %	0.66	0.55	0.44
Threonine, %	0.76	0.61	0.47

[a] Premix composition per kilogram of diet: Weaner and gestation diets - Vitamin A 10,000 IU, vitamin D 1,000 IU, vitamin E 80 IU, vitamin K 2 mg, vitamin B12 30 µg, riboflavin 12 mg, niacin 40 mg, pantothenic acid 25 mg, choline 1,000 mg, biotin 250 µg, folic acid 1,600 µg, ethoxyquin 5 mg, iron 150 mg, manganese 12 mg, zinc 120 mg, copper 20 mg, iodine 200 µg, selenium 300 µg. Grower diet - Vitamin A 5,000 IU, vitamin D 500 IU, vitamin E 40 IU, vitamin K 2 mg, vitamin B12 30 µg, riboflavin 12 mg, niacin 40 mg, pantothenic acid 25 mg, choline 300 mg, biotin 150 µg, ethoxyquin 5.0 mg, iron 150 mg, manganese 12 mg, zinc 100 mg, copper 20 mg, iodine 200 µg, selenium 300 µg.

Table 3.2 Feed intake, body weight, body weight change, backfat thickness, and backfat change of HH, HR, and RH gilts at d 0, d 7, d 15 and at estrus (LSM ± SEM)

Parameter	HH[1]	HR[1]	RH[1]
Feed intake, kg[2]			
d 1-d 7	3.0 ± 0.1	2.9 ± 0.1	2.2 ± 0.1
d 8-d 15	3.5 ± 0.1	2.7 ± 0.1	3.4 ± 0.1
d 16-estrus	3.0 ± 0.1	3.0 ± 0.1	2.8 ± 0.1
Body weight, kg			
d 0	133.0 ± 2.1	132.0± 2.3	129.8 ± 2.1
d 7	137.0 ± 0.7^{a}	136.8± .8^{a}	133.7 ± 0.7^{b}
d 15	145.4 ± 0.6^{c}	142.3± .6^{d}	143.0 ± 0.6^{d}
Estrus	146.2 ± 0.9	144.7±1.0	143.7 ± 0.9
Body weight change, kg			
d 1-d 7	5.6 ± 0.7^{e}	5.6 ± 0.8^{e}	2.5 ± 0.7^{f}
d 8-d 15	8.5 ± 0.4^{g}	5.5 ± 0.5^{h}	9.4 ± 0.5^{g}
d 16- estrus	0.9 ± 0.7	2.4 ± 0.7	0.6 ± 0.7
Backfat, mm			
d 0	12.8 ± 0.4	12.0 ± 0.4	12.2 ± 0.4
d 7	13.0 ± 0.2	13.1 ± 0.3	12.5 ± 0.2
d 15	14.1 ± 0.2^{x}	13.6 ±	13.2 ± 0.2^{y}
Estrus	14.2 ± 0.2^{w}	13.5 ±	13.1 ± 0.2^{z}
Backfat change, mm			
d 1-d 7	0.6 ± 0.2	0.8 ± 0.3	0.2 ± 0.2
d 8-d 15	1.0 ± 0.3	0.4 ± 0.3	0.7 ± 0.3
d 16-estrus	0.1 ± 0.2	-0.02 ± 0.2	-0.1 ± 0.2

[1] HH: gilts fed a high plane of nutrition (2.8 X maintenance) throughout the estrous cycle; HR: gilts fed a high plane of nutrition (2.8 X maintenance) from d 1 to d 7, restricted (2.1 X maintenance) from d 8 to d 15 and then fed a high plane again from d 16 until onset of estrus; RH gilts restricted

(2.1 X maintenance) from d 1 to d 7, then fed a high plane (2.8 X maintenance) from d 8 until onset of estrus.

[2] Feed offered, kg: d1 to d 7 - 3.5 ± 0.04, 3.5 ± 0.04, 2.6 ± 0.04; d 8 to d 15 – 3.6 ± 0.04, 2.7 ± 0.06, 3.6 ± 0.04; d 16 to estrus - 3.8 ± 0.04, 3.8 ± 0.04, 3.8 ± 0.04, respectively for HH, HR and RH gilts.

[a,b,c,d,e,f,g,h,x,y,w,z] LSMeans within rows with different superscripts differ ($P < 0.05$).

Table 3.3 Reproductive characteristics at day 28 of gestation in gilts subjected to different feed regimens before mating (LSM ± SEM)

Treatment (n)	Ovulation rate (CL #)	No. live embryos	Embryo size, mm	Embryo survival, %
HH[1] (22)	17.1 ± 0.6	14.3 ± 0.9	22.5 ± 0.5	83.6 ± 4.3^a
HR[1] (19)	18.5 ± 0.6	12.8 ± 1.0	21.7 ± 0.5	68.3 ± 4.8^b
RH[1] (21)	17.7 ± 0.6	14.7 ± 1.0	23.0 ± 0.5	81.7 ± 4.5^a

[1] HH: gilts fed a high plane of nutrition (2.8 X maintenance) throughout the estrous cycle; HR: gilts fed a high plane of nutrition (2.8 X maintenance) from d 1 to d 7, restricted (2.1 X maintenance) from d 8 to d 15 and then fed a high plane again from d 16 until onset of estrus; RH gilts restricted (2.1 X maintenance) from d 1 to d 7, then fed a high plane (2.8 X maintenance) from d 8 until onset of estrus.

[a,b] LSMeans within columns with different superscripts differ, P = 0.038 (analysis based on arcsine transformed data).

Figure 3.1 Plasma progesterone concentrations (LSM ± SEM) of gilts at 36, 48, 72 and 96 h after onset of estrus (● HH, ■ HR, ▲ RH). Treatment groups: HH: gilts fed a high plane of nutrition (2.8 X maintenance) throughout the estrous cycle; HR: gilts fed a high plane of nutrition (2.8 X maintenance) from d 1 to d 7, restricted (2.1 X maintenance) from d 8 to d 15 and then fed a high plane again from d 16 until onset of estrus; RH gilts restricted (2.1 X maintenance) from d 1 to d 7, then fed a high plane (2.8 X maintenance) from d 8 until onset of estrus. * Difference between LSMeans $P < 0.05$.

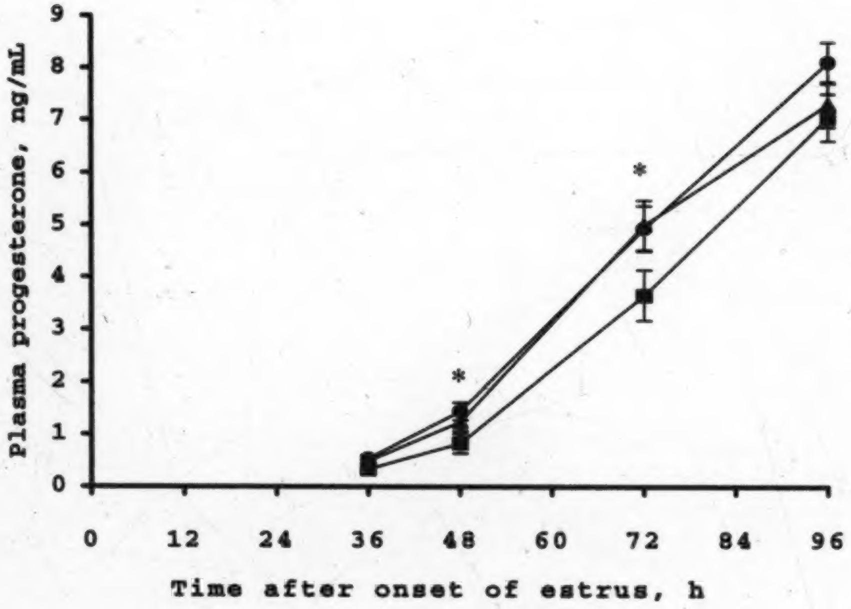

Plasma progesterone, ng/mL
0
1
2
3
4
5
6
7
8
9
0
12
24
36
48
60
72
84
96
Time after onset of estrus, h
*
*

Figure 3.2 Relationship between residual embryo size (Y) and residual allantochorionic area (X) after correction for block, treatment and day. Embryo size and allantochorionic area are positively correlated (Plot of residuals: Embryo size = 0.0003 x (area) + 18.35, r = 0.28, P = 0.03).

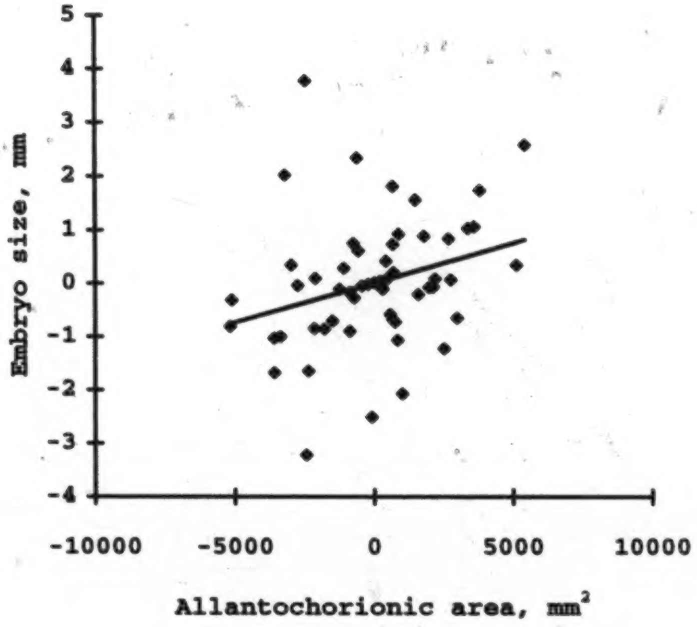
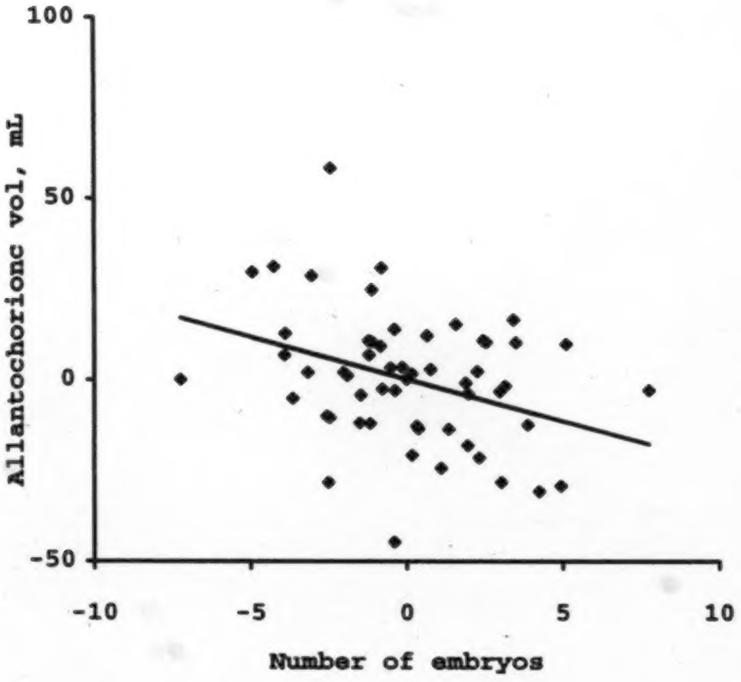

Embryo size, mm
5
4
3
2
1
0
-1
-2
-3
-4
-10000
-5000
0
5000
10000
Allantochorionic area, mm²

Figure 3.3 Relationship between residual allantochorionic volume (Y) and residual number of embryos (X) after correction for block, treatment and day. Allantochorionic volume and number of embryos are negatively correlated (Plot of residuals: Placental volume = -4.317 x (number) + 207.55, r = -0.39, P = 0.002).


100
50
0
-50
Allantochorionc vol, mL
-10
-5
0
5
10
Number of embryos

CHAPTER 4

PATTERNS OF FEED INTAKE DURING THE ESTROUS CYCLE AND THE DEVELOPMENTAL COMPETENCE OF PORCINE EMBRYOS CULTURED *IN VITRO*

4.1 Introduction

Optimal reproductive performance is an important goal in livestock production systems. In the swine industry, the number of pigs weaned per sow per year is a key measure of reproductive efficiency. As gilts are an important component of the breeding herd, it is essential to improve fertility in these animals, emphasizing genetics, nutrition and management practices. Given the variation in these factors, the development of strategies to maximize litter size is challenging.

The variance of embryo survival in the pig is large, and on average only 75% of blastocysts recorded on day 9 of pregnancy survives until day 25 (Pope and First, 1985). A high proportion of loss occurs around the time of maternal recognition of pregnancy and implantation (days 12 to 18), when the more advanced embryos secrete estradiol, which acts as a signal to prevent luteolysis (Bazer and Thatcher, 1977) and promotes changes in the uterine milieu (Davis and Blair, 1993).

The developing ovarian follicle provides the maturational environment for the oocyte, and differences in follicular maturation are associated with differences in the ability of these follicles to support oocyte maturation (Hunter and Wiesak, 1990; Xie et al. 1990; Ding and Foxcroft, 1994). In turn, the maturational state of the oocyte immediately before ovulation may contribute to differences in embryonic survival, as oogenesis directs embryogenesis (Pope et al., 1990). Studies conducted by Xie et al. (1990) established that late-ovulating follicles give rise to less developed embryos on day 4 of gestation in gilts. In addition, it has been demonstrated that plasma steroid concentrations in the peri-ovulatory period can affect embryo survival (Miller et al., 1977; Pope, 1994; Dziuk, 1987; Jindal et al. 1997). Thus, there is evidence that the physiological state of the follicle, and the maturational state of the oocyte, give rise to embryos that exhibit different abilities to develop within the same uterine environment.

Experimental paradigms involving manipulation of feed intake in the peri-ovulatory period in gilts (Ashworth, 1991; Pharazyn et al., 1991; Jindal et al., 1996) or during lactation in sows (Baidoo et al., 1992; Zak et al., 1997a) influence embryo survival, independent of effects on ovulation rate. Additionally, Zak et al. (1997b) and Yang et al. (2000) demonstrated that the status of follicles in the pre-ovulatory pool, the rate of maturation of oocytes obtained from these follicles, and the ability of follicular fluid from these follicles to support in vitro maturation of standardized pools of oocytes, were affected by the nutritional history of the lactating sow. Indeed, recent studies conducted in sheep by McEvoy et al. (1995a, b) demonstrated that the manipulation of feed intake during the peri-ovulatory period could affect the developmental competence of early-fertilized oocytes *in vitro*. Interestingly, in the only comparable study published before the start of the present work, a series of experiments with reciprocal embryo transfer suggested that nutritional effects on the uterus were key mediators of embryo survival (Bazer et al., 1968); however, data from a relatively small number of gilts was used to establish this hypothesis.

A previous study in our laboratory (Almeida et al., 2000) used the cyclic gilt as a model to study the interactions between nutrition and reproduction. In this model, gilts were fed either high plane throughout the estrous cycle (HH), or feed intake was restricted during the first (RH) or second (HR) week of the cycle. Although pattern of feeding had no effect on ovulation rate, embryonic survival at d 28 of pregnancy in the HR gilts was significantly reduced compared to RH and HH gilts, and HR gilts also had lower plasma progesterone concentrations at 48 and 72 h after onset of estrus. The objective of the present study was to use the same gilt model to evaluate the mechanisms that mediate metabolic effects in the pre-ovulatory period on post-ovulatory reproductive function, and specifically to clearly establish whether previous nutritional treatments affected the developmental competence *in vitro* of oocytes recovered immediately after fertilization. Other aspects of the study, to assess associated treatment effects on oviductal protein synthesis and secretion, as well as on uterine and oviductal gene expression at sequential time points after ovulation, will be reported elsewhere.

4.2 Materials and Methods

4.2.1 Animals

The experiment was conducted at the Swine Research Unit of the University of Alberta, in barns with a totally controlled environment, using 19 pairs of littermate gilts (Pig Improvement Canada Ltd, Camborough x Canabrid terminal line) in their second estrous cycle. The procedures of selection, pre-treatment and experimental treatments of gilts were performed as in our previous study (Almeida et al., 2000), and data on body weight and backfat at 160 days of age and body weight, backfat and age at pubertal estrus are shown in Appendix 4.1 and 4.2. Weekly growth rate of gilts are presented in Appendix 4.1. However, the treatment comparison was limited to the RH and the HR feeding regimens. Therefore, in the present study, littermate gilts were allocated to one of the following treatments: feed restriction to 2.1 X energy requirements for maintenance from d 1 to 7 of the cycle, and then fed 2.8 X maintenance from d 8 until onset of estrus (RH) or fed 2.8 X maintenance from d 1 to 7, restricted to 2.1 X maintenance from d 8 to 15 and, refed at 2.8 X maintenance from d 16 until onset of estrus (HR). All gilts were fed a wheat-barley-soybean grower diet, which was nutritionally balanced in terms of amino acids, vitamins and minerals to meet NRC (1988) recommended nutritional requirements. Body weight and backfat thickness at P_2 (Renco Lean-Meter, Renco Corporation, Minneapolis, MN, USA) were measured in all animals at d 0 (onset of second estrus), 7, 15 and at the onset of the third estrus.

Of the 38 gilts initially allocated to treatment, two (one HR and one RH) did not show behavioral estrus after treatment, one (HR) was sick during treatment and six (two HR and four RH) had embryos with more than two cells at collection. Data from these animals were excluded from the final analysis, and treatment effects on developmental competence were therefore based on 15 HR and 14 RH gilts.

All experimental procedures were carried out in accordance with the guidelines of the Canadian Council for Animal Care and under authorization from the Faculty Animal Policy and Welfare Committee of the University of Alberta.

4.2.2 Insemination and embryo recovery

Starting at d 19 of the second estrous cycle, gilts were checked for estrus every 6 h (0600, 1200, 1800 and 2400) using the back pressure test during periods of fence line contact with mature vasectomized boars. Gilts were artificially inseminated 12 and 24 h after the first observed standing estrus with pooled semen (3×10^9 spermatozoa/dose) from the same group of boars (Alberta Swine Genetics Corporation, Leduc, AB, Canada) specifically designated for this experiment. Time of ovulation was monitored using transcutaneous ultrasonography (Pie Medical Scanner 200, model 41480, Can Medical, Kingston, Ontario, Canada), using a 5.0 – 7.5 MHz multiple angle transducer to scan for the presence of pre-ovulatory follicles. Gilts were scanned every 6 h, beginning 24 h after onset of standing estrus, until completion of ovulation. During each scanning, follicle diameter was recorded. Time of ovulation was defined as the time of the first scanning when no presumptive ovulatory follicles were seen less three hours. Blood samples were taken by acute venipuncture at ovulation, 12 h after ovulation, 48 h after onset of standing heat and at surgery for progesterone assay. In the 48 h sample after onset of standing heat, as heat detection was performed at 6-h intervals, the timing was adjusted so that it could be exactly the same time point as in the first experiment, when heat detection was performed at 12-h intervals.

Surgeries were performed under general anesthesia 12 to 20 h after ovulation to recover early-fertilized oocytes. The surgical procedure included laparotomy and exposure of the uterine horns, oviducts and ovaries. Ovulation rates were recorded and each oviduct was flushed twice using 5 mL of Dulbecco's Phosphate Buffered Saline (DPBS, Sigma Chemical Company, St. Louis, MO, USA), previously warmed at 39° C. Flushings were collected in sterile Falcon dishes (Fisher Scientific, St. Louis, MO, USA) and transported to the laboratory in a Styrofoam box containing a tray and flasks filled with warm water to avoid cooling of the recovered oocytes.

Falcon dishes containing 2 mL of NCSU-23 culture media supplemented with 4 mg/mL BSA (Sigma Chemical Co., St. Louis, MO, USA, catalogue # 8022) were prepared and left in incubators to warm and pre-gas at least 2 h before surgery. Embryos were immediately transferred into the culture dishes and incubated under standard conditions of 39° C and 5% CO_2. Embryo development was observed once a day for seven days using a dissecting microscope (Wild M3, Wild Heerbrugg, Switzerland) and 16X and 40X magnification to determine developmental potential. Indication of fertilization (presence of sperm heads on the zona pellucida) and abnormal features (cells dividing unevenly) were observed under an inverted stage phase-contrast microscope at 400X magnification (Nikon Corp., Tokyo, Japan). Because embryos collected at later stages are more likely to develop to the morula and blastocyst stages, gilts that had all embryos with more than two cells at collection were excluded from the study.

4.2.3 Radioimmunoassay

Plasma progesterone concentrations were determined using an established radioimmunoassay (Coat-a-Count Progesterone, Diagnostic Products Corporation, Los Angeles, CA, USA), previously validated for use with porcine plasma without extraction (Mao and Foxcroft, 1998). The sensitivity of the assay, defined as 97 % of total binding, was 0.098 ng/mL. The intra- and inter-assay CV were 9.3 and 12.3 %, respectively.

4.2.4 Statistical analysis

Data were analyzed as a randomized complete block design, each block consisting of two littermates representing each treatment. Treatment effects on ovulation rate, fertilization rate, body weight and backfat changes, embryo developmental competence and progesterone concentrations after ovulation were analyzed using the general linear model (GLM) procedure of the Statistical Analysis

System (SAS, 1990). The analysis of body weight and backfat changes during treatment included the effects of block and treatment in the model, with body weight and backfat at d 0 of the treatment cycle as covariates. For evaluation of treatment effects on embryo developmental competence *in vitro*, data were arc sin transformed prior to analysis. As progesterone concentrations did not present a normal distribution, these data were log transformed prior to analysis. The complete model included treatment, block, and gilt within treatment by block interaction, with the latter used as the error term. In the event that significant treatment effects were established, comparisons between least square means were performed using the probability of differences (pdiff), adjusted by Tukey-Kramer (SAS, 1990).

4.3 Results

4.3.1 Body weight and backfat changes

Feed intake, body weight and weight change, backfat thickness at P_2 and backfat change of gilts are summarized in Table 4.1.

Both RH and HR gilts had a similar growth rate during the period of d 1 to d 7. The HR group had a lower growth rate ($P = 0.0015$) during the period of feed restriction (d 8 to d 15) compared to their RH counterparts. There was no difference ($P > 0.05$) in growth rate between groups from d 16 until onset of estrus probably due to their low feed intake in this period. Backfat changes were not different among groups during the experimental period. A significant litter effect was observed for body weight at d 0 ($P = 0.0489$) and at estrus onset ($P = 0.0199$) and for backfat at d 15 ($P = 0.0266$) and at estrus onset ($P = 0.0327$).

4.3.2 Embryo developmental competence

Reproductive characteristics of gilts (Table 4.2) showed that the number of ovulations and the number of embryos recovered did not differ between treatments ($P > 0.05$). Consistent with our previous study (Almeida et al., 1999), a significant litter effect was observed for ovulation rate among gilts ($P = 0.039$). Additionally, embryo recovery rate was similar for both treatments, and fertilization rate tended to be higher ($P = 0.056$) in the RH group than in the HR group.

The development of embryos recovered from each treatment group is summarized in Figure 4.1. There was a gradual decrease in the percentage of embryos reaching successive stages of early development and a marked decrease in the percentage of embryos making the transition from morula to blastocyst. No treatment effects were observed on the development of embryos to the 4-8 cell and morula stages, nor on the percentage of blastocysts obtained at 96 and at 144 h of culture. However, a significant litter effect ($P = 0.01$) was observed for the percentage of blastocysts at 96 h of culture.

4.3.3 Plasma progesterone concentrations

Previous nutritional treatments did not affect ($P > 0.05$) progesterone concentrations after ovulation (Table 4.3).

4.4 Discussion

Consistent with the data reported by Almeida et al. (2000), the patterns of feed intake imposed during the estrous cycle resulted in differential patterns of body weight change in RH and HR gilts. During the period of feed restriction (d 8 to 15), gilts in the HR treatment group gained less than their counterparts fed a high plane of nutrition (RH). However, similar growth rates observed during d 1 to 7, when RH gilts were restricted, can be explained by the relatively low feed intake of HR gilts in the immediate post-estrous period (as shown in Table 4.1), confirming the observations of Williams et al. (1974) that food availability is the most important environmental factor that can affect growth. Furthermore, Booth et al. (1994) reported that gut fill clearly played a role in live weight variation and would tend to mask initial effects of feed restriction and exaggerate effects of an increase in feed intake. The lack of an effect on backfat change is likely due to the modest level of feed restriction used (2.1 versus 2.8 X maintenance) which allowed the animals to continue growing, even during periods of restricted feeding.

In the present experiment, our aim was to culture preimplantation porcine embryos to the unexpanded blastocyst stage in a simple culture media, which has been reported to support the development of one- two-cell embryos to the blastocyst stage (see for review Petters and Wells, 1993). Therefore, the culture media used was the NCSU-23 (North Caroline State University-23), which has been successfully used by others (Illera et al., 1992; Torres and Rath, 1992; Rath et al., 1995; Macháty et al., 1998; Long et al., 1999). Among the components of this culture media, it has been reported that the amino acids taurine and hypotaurine enhance the development of pig embryos in vitro (Petters and Reed, 1991; Reed et al., 1992; Petters and Wells, 1993). Even though the media was considered appropriate for our study, the data in Figure 4.1 indicate an "*in vitro* developmental block", as shown by the lower percentage of embryos reaching the blastocyst stage (around 60%) compared to those reaching the morula stage (around 85%). In the present study, the culture media was supplemented with BSA (Sigma A-8022) fraction V as a protein source. In a previous study conducted by Dobrinsky et al. (1996), embryos cultured in BSA fraction V showed reduced developmental competence, which may be the result of contaminants in the preparation of BSA-V, and these detrimental effects may outweigh the positive components provided in BSA. Pemble and Kaye (1986) suggested that protein such as BSA may be essential in culture media as it can be taken up by the embryo and broken down to provide energy substrates and amino acids for metabolic and anabolic processes. Thomassen (1989) suggested that BSA fraction V could have varying levels of contaminants that can be stimulatory or inhibitory to cell proliferation. Although, fatty-acid free preparations of BSA could have some or all contaminants removed by the extraction procedures, this may vary from lot to lot. Furthermore, the addition of fetal calf serum before the morula stage has been reported to accelerate development just after this blocking stage (Koo et al., 1997; Van Langendonckt et al., 1997). Therefore, the low percentage of embryos reaching the blastocyst stage in our study may be explained by the use of BSA fraction V as the protein source. However, we believe that the *in vitro* methodology used would still allow the possibility of measuring treatment effects on the developmental competence of recovered oocytes.

All the key factors that determine whether an oocyte develops to the blastocyst stage after fertilization are not yet fully understood. Various bovine studies have highlighted the fact that the fastest developing embryos *in vitro* are more likely to develop to the morula and blastocyst stages (Plante et al., 1994; Itagaki et al., 1997; Kubisch et al., 1998). Moreover, those blastocysts that form early appear to have a greater likelihood of providing live offspring after transfer (Hasler et al., 1995). Lonergan et al. (1999) showed that time of first cleavage had a major influence on the probability of an embryo developing to the blastocyst stage, but once this stage was attained, subsequent developmental characteristics were unrelated to the time of first cleavage. Therefore, effects of previous nutritional treatment on early embryonic survival could be mediated by effects on early stages of embryonic development. In turn, this might be related to the quality of the oocyte before fertilization.

In our study, higher fertilization rate in the RH gilts, provided with a high plane of nutrition (2.8 X maintenance) during the late luteal phase of the cycle, would be consistent with the concept of higher quality oocytes in this group, although indirect effects of nutritional treatments on oviductal function and sperm maturation may also be a factor. Successful fertilization depends mainly on the time of insemination or mating relative to ovulation (Wabersky et al., 1994; Soede et al., 1995). If insemination is performed either too early, or too late, which is possible in pigs since estrus may first be observed as long as 60 h before, and extend for up to 24 h after ovulation, a deleterious influence upon fertility is seen as: 1) a reduced conception rate, 2) a lower incidence of normal fertilization, and 3) an increase in embryonic loss (Hunter, 1977). As shown in Table 4.2, the interval from the last insemination to ovulation in the present study is consistent in both treatment groups, suggesting that previous nutritional regimens affected fertilization rate by some other mechanism than an effect on ovulation.

The absence of treatment effect on developmental competence of early-fertilized oocytes to the blastocyst stage is in agreement with the recent comparable findings of Graham et al. (1999), in which no difference was observed in the percentage of blastocyst formation in gilts at first estrus fed ad libitum and gilts at third estrus fed either ad libitum or restricted. In the earlier studies of Bazer et al. (1968), the use of reciprocal embryo transfer between gilts subjected to different nutritional regimens, also led to the conclusion that nutritional effects on the uterine environment, rather than the inherent developmental competence of the oocyte, mediate nutritional effects on pre-natal survival. In contrast, in studies conducted in sheep by McEvoy and coworkers (1995b) in which nutrition was manipulated in the pre-ovulatory period, embryonic development and viability in vitro were compromised in super-ovulated ewes. In recent studies conducted in bovine (Nolan et al., 1998), again nutrition manipulation in the peri-ovulatory period resulted in improved embryo quality, as evident from the increased number of blastocysts formed and higher numbers of blastomeres. On the other hand, Pope (1994) suggested that although the immediate impact of the spread in ovulation time may appear limited, the complex interactions between embryos of different maturity with the uterine environment greatly amplifies developmental differences in this transitional stage of development on day 11 to 12, and places the smaller embryos at risk. It is possible, therefore, that effects of nutritional treatments on the inherent developmental potential of the embryo may not be expressed during the limited period of *in vitro* culture used.

The role of peri-ovulatory progesterone as a possible mediator of nutritionally induced effects on embryonic survival has been studied in ewes and gilts (Ashworth et al., 1989, Ashworth et al., 1991; Jindal et al., 1997). Ashworth et al. (1991) and Jindal et al. (1997) demonstrated in gilts that progesterone injections in the early stages of pregnancy could counteract a nutritionally induced increase in embryonic loss. Studies in ewes have also suggested that the level of priming progesterone, modulated by pre-ovulatory nutrition, influenced embryo survival through direct effects on the developing oocyte (McEvoy et al., 1995a). In subsequent studies, McEvoy et al. (1995b) reported that the provision of supplementary progesterone to ewes on a high plane of feeding during the pre-ovulatory priming phase elevated plasma progesterone and enhanced subsequent ovum development. This confirms the importance of monitoring the patterns of progesterone secretion at critical times in early pregnancy (Foxcroft, 1997) and also raises the additional possibility, previously discussed by Hunter and Wiesak (1990), that differences in follicular maturation before ovulation may be reflected in subtle but physiologically important differences in progesterone secretion in early pregnancy. Moreover, Savio et al. (1993) and Wehrman et al. (1993) suggested that low pre-ovulatory progesterone concentrations lead to sub-optimal oocyte maturation and reduced fertility in cattle.

Therefore, there seems to be increasing evidence that a number of the effects of nutrition on embryonic survival are mediated by progesterone. However, the precise mechanisms involved need to be elucidated. In our previous study (Almeida et al., 2000), the HR gilts had lower embryonic survival and also lower progesterone concentrations at 48 and 72 h after onset of estrus compared to their HH and RH counterparts. If a difference in plasma progesterone is an essential mediator of nutritional effects on early embryonic development, then similar progesterone concentrations in the post-ovulatory period in the present study could provide the basis for similar embryo development capabilities between treatments. There would, however, be no obvious explanation for the apparent inconsistency in a treatment effect on progesterone concentrations in early pregnancy between our previous study (Almeida et al., 2000) and the present study.

In conclusion, our results indicate that either previous nutritional treatment does not impact the early developmental competence of fertilized oocytes and nutritional effects on embryonic survival to d 28 are mediated by other mechanisms, or differences in developmental competence of oocytes are not expressed up to the unexpanded blastocyst stage. However, previous nutritional treatment did affect the fertilization of recovered oocytes. Evidence for the role of progesterone as an essential mediator of nutritionally induced effects on embryo survival is equivocal.

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Table 4.1 Feed intake, body weight, body weight change, backfat thickness and backfat change of RH and HR gilts at d 0, d 7, d 15 and at estrus (LSM ± SEM)

Parameter	RH[1]	HR[1]
Feed intake, kg[2]		
d 1- d 7	2.4 ± 0.06	3.0 ± 0.06
d 8-d 15	3.6 ± 0.05	2.7 ± 0.05
d 16-Estrus	2.9 ± 0.15	3.0 ± 0.16
Body weight, kg		
d 0	134.2 ± 2.0	130.8 ± 2.0
d 7	137.0 ± 0.9	139.0 ± 0.9
d 15	147.2 ± 0.8 ^{a}	144.4 ± 0.8 ^{b}
Estrus	148.8 ± 0.6	148.0 ± 0.7
Body weight change, kg		
d 1- d 7	4.5 ± 0.9	6.3 ± 0.9
d 8-d 15	10.2 ± 0.8 ^{c}	5.5 ± 0.8 ^{d}
d 16-Estrus	1.6 ± 1.2	1.4 ± 1.4
Backfat, mm		
d 0	11.7 ± 0.4	10.9 ± 0.4
d 7	13.0 ± 0.3	12.5 ± 0.3
d 15	13.3 ± 0.2	12.8 ± 0.2
Estrus	13.3 ± 0.2	13.0 ± 0.2
Backfat change, mm		
d 1- d 7	1.7 ± 0.2	1.2 ± 0.2
d 8-d 15	0.3 ± 0.2	0.3 ± 0.2
d 16-Estrus	-0.01 ± 0.3	0.15 ± 0.3

[1] RH: gilts restricted (2.1 X maintenance) from d 1 to d 7, then fed a high plane (2.8 X maintenance) from d 8 until onset of estrus; HR: gilts fed a high plane (2.8 X maintenance) from d 1 to d 7, restricted (2.1 X maintenance) from d 8 to d 15 and then fed high plane again from d 16 until onset of estrus.

[2] Feed offered (kg): d 1 to d 7 – 2.6 ± 0.04, 3.5 ± 0.04; d 8 to d 15 – 3.7 ± 0.04, 2.7 ± 0.04; d 16 to estrus – 3.8 ± 0.04, 3.8 ± 0.04, respectively for RH and HR gilts.

[a,b,c,d] LSMeans within rows with different superscripts differ ($P < 0.05$).

Table 4.2 Reproductive characteristics of gilts on treatment (LSM ± SEM)

Parameter	RH[1]	HR[1]
N	14	15
Ovulation rate	17.1 ± 0.5	17.2 ± 0.6
Number of embryos recovered	14.8 ± 0.6	14.4 ± 0.6
Recovery rate, %	85.0 ± 2.8	86.7 ± 3.0
Fertilization rate, %	99.5 ± 1.4 ^{x}	95.4 ± 1.3 ^{y}
Time of ovulation, h	42.8 ± 1.3	45.4 ± 1.4
Interval from last insemination to ovulation, h	14.0 ± 1.5	14.7 ± 1.7

[1] RH: gilts restricted (2.1 x maintenance) from d 1 to d 7, then fed a high plane (2.8 x maintenance) from d 8 until onset of estrus; HR: gilts fed a high plane (2.8 x maintenance) from d 1 to d 7, restricted (2.1 x maintenance) from d 8 to d 15 and then fed high plane again from d 16 until onset of estrus.

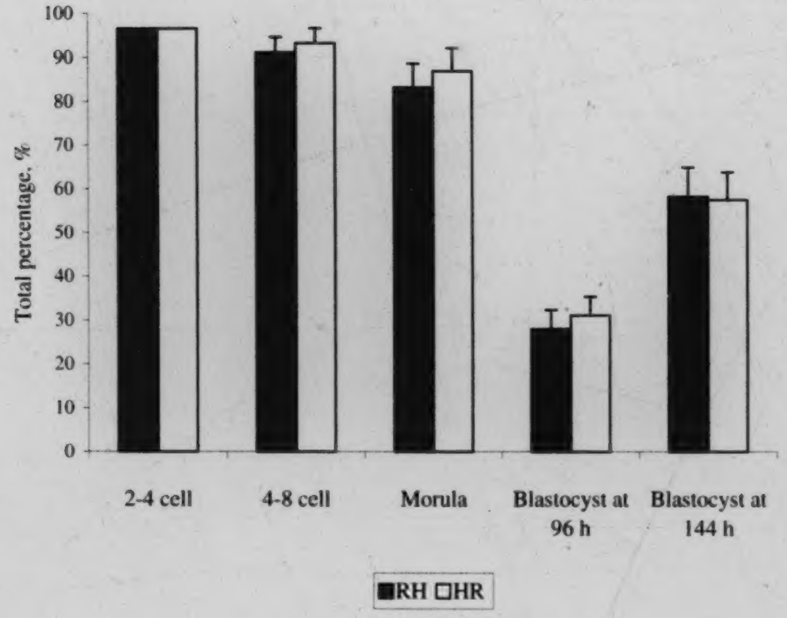
^{x,y} difference at P = 0.056 between treatment least square means (analysis based on arcsin transformed data).

Table 4.3 Plasma progesterone concentrations (ng/mL) at ovulation, 12 h after ovulation, 48 h after onset of estrus and at surgery (LSM ± SEM)

Time	RH[1]	HR[1]
Ovulation	0.65 ± 0.19	0.58 ± 0.18
12 h after ovulation	1.39 ± 0.28	1.79 ± 0.26
48 h after onset of estrus	1.28 ± 0.27	1.04 ± 0.25
Surgery	1.87 ± 0.31	1.98 ± 0.29

[1] RH: gilts restricted (2.1 X maintenance) from d 1 to d 7, then fed a high plane (2.8 X maintenance) from d 8 until onset of estrus; HR: gilts fed a high plane (2.8 X maintenance) from d 1 to d 7, restricted (2.1 X maintenance) from d 8 to d 15 and then fed high plane again from d 16 until onset of estrus.

Figure 4.1 Percentage of fertilized oocytes recovered 12 to 20 h after ovulation reaching successive stages of development during culture in vitro for 144 h.


Total percentage, %
100
90
80
70
60
50
40
30
20
10
0
2-4 cell
4-8 cell
Morula
Blastocyst at 96 h
Blastocyst at 144 h
■RH ☐HR

CHAPTER 5

EFFECTS OF DIFFERENT PATTERNS OF FEED RESTRICTION AND INSULIN TREATMENT DURING THE LUTEAL PHASE ON REPRODUCTIVE, METABOLIC AND ENDOCRINE PARAMETERS IN CYCLIC GILTS

5.1 Introduction

The influence of nutrition on reproduction has been extensively investigated and it is now generally accepted that reproductive capacity cannot be optimized without adequate nutrition. There is increasing evidence that changes in metabolic fuel availability and associated endocrine status are important determinants in the signaling of metabolic status to the reproductive axis (I'Anson et al., 1991; Foxcroft, 1992; Wade and Schneider, 1992; Wade et al., 1996). Understanding the mechanisms linking these two aspects of physiology is of great interest for the improvement of fertility in animals, leading to optimal reproductive efficiency.

Results on the phenomenon of 'flushing' reported in gilts (Flowers et al., 1989; Beltranena et al., 1991) demonstrated that the nutritional stimulation of ovulation rate is effected via central (hypophyseal) effects on LH pulsatility and local (ovarian) mechanisms. Both central (Cosgrove et al., 1993; Booth et al., 1996) and local mechanisms (Cosgrove et al., 1992; Charlton et al., 1993) have also been identified in studies using feed restricted prepubertal gilt models and in studies with cyclic gilts (Armstrong and Britt, 1987; Cox et al., 1987). However, the relative importance of central and local mechanisms underlying nutrition-reproduction interactions has yet to be satisfactorily addressed.

There is increasing evidence to suggest a positive relationship between insulin and ovarian function in swine. Insulin administration during the follicular phase increased ovulation rate (Cox et al., 1987) and decreased follicular atresia (Matamoros et al., 1990, 1991) in cyclic gilts, and was not associated with any consistent increase in gonadotropin secretion. Conversely, withdrawal of insulin from diabetic gilts during the luteal phase decreased ovulation rate and increased the rate of follicular atresia (Cox et al., 1994). Additionally, pituitary LH stores were depleted, and estrous cycles were interrupted, in these animals (Cox et al., 1994; Angell et al., 1996). In sows, Tokach et al. (1992) and Koketsu et al. (1996) demonstrated that circulating concentrations of insulin and glucose and the number of LH pulses during lactation were greater in sows with shorter weaning to estrus intervals. Compared to sows fed to appetite, Zak et al. (1997a) observed a decrease in ovulation rate and an increase in weaning to estrus interval in primiparous sows subjected to feed restriction either during weeks 1 to 3, or during week 4 of lactation; whereas embryo survival was only lower in sows restricted during week 4. Subsequently, Zak et al. (1997b) and Yang et al. (2000a) demonstrated that the status of follicles in the pre-ovulatory pool, the rate of maturation of oocytes obtained from these follicles, and the ability of follicular fluid from these follicles to support in vitro maturation of standardized pools of oocytes, were affected by the previous nutritional history of the lactating sow.

A previous study in our laboratory (Almeida et al., 2000) used short-term changes in feed intake in cyclic gilts to further study the interactions between nutrition and reproduction. In this model, littermate gilts were fed either at a high plane throughout the estrous cycle (HH), or feed intake was restricted during the first (RH) or second (HR) week of the cycle. Although pattern of feeding had no effect on ovulation rate, embryonic survival at d 28 of pregnancy in the HR gilts was significantly reduced compared to RH and HH gilts, and HR gilts also had lower plasma progesterone concentrations at 48 and 72 h after onset of estrus. Moreover, in a subsequent study in our laboratory using the same experimental paradigm (see Chapter 4), different patterns of feed intake during the previous estrous cycle affected fertilization rate of oocytes recovered immediately after ovulation, without any detrimental effect on oocyte developmental competence *in vitro*. Based on these results, and on the literature reviewed above, the objectives of the present study were to continue to use this established cyclic gilt model to 1) extend our observations on potential effects of previous nutritional treatment on developmental competence of early fertilized oocytes *in vitro*; 2) study responses to insulin treatment during the period of feed restriction in the late luteal phase which has deleterious effects on subsequent fertility, and 3) establish the metabolic and endocrine status of gilts during treatment and during the subsequent peri-estrus period. Other aspects of the study, to assess associated treatment effects on oviductal protein synthesis and secretion, on uterine and oviductal gene expression at sequential time points after ovulation, and on molecular regulation of early luteal function, will be reported elsewhere.

5.2 Materials and Methods

5.2.1 Animals

The experiment was conducted at the Swine Research Unit of the University of Alberta, in barns with a totally controlled environment, using 19 trios of littermate gilts (Pig Improvement Canada Ltd, Camborough x Canabrid terminal line) in their second estrous cycle. Selection, pre-treatment and management of nutritional treatments of gilts were performed as in our previous study (Almeida et al., 2000), and data on body weight and backfat at 160 days, as well as age, body weight and backfat at pubertal estrus are shown in Appendix 5.1 and 5.2. Weekly growth rate of gilts are shown in Appendix 5.1. However, the nutritional treatments were limited to the RH and the HR feeding regimens. A second group of HR gilts received injections of long acting insulin subcutaneously behind the ear at the time of feeding (0.8 I.U./kg body weight, Iletin® Lente Insulin Pork, Elli Lilly, Indianapolis, IN, USA), using flexible butterfly catheters (21G ¾ needle, Becton Dickinson, Franklin, New Jersey, USA) and without any restraint, during their period of feed restriction (HR+I). Therefore, in the present study, littermate gilts were allocated to one of the following treatments: feed restriction to 2.1 X energy requirements for maintenance from d 1 to 7 of the cycle, and then fed 2.8 X maintenance from d 8 until onset of estrus (RH), or fed 2.8 X maintenance from d 1 to 7, restricted to 2.1 X maintenance from d 8 to 15, and then refed at 2.8 X maintenance from d 16 until onset of estrus, with (HR+I) or without (HR) insulin treatment from d 8 to 15. All gilts were fed a wheat-barley-soybean grower diet, which was nutritionally balanced in terms of amino acids, vitamins and minerals to meet NRC (1988) recommended nutritional requirements. To avoid severe hypoglycemia, and based on preliminary studies to titrate the hypoglycemic effects of insulin treatment, insulin treated gilts were given 30 mL of corn syrup in the feed, which corresponded to an increment of about 300 kcal in their daily energy intake. Blood glucose was monitored in all gilts 3 h after the afternoon feeding on d 8, 12 and 15 using a glucometer (One Touch II, Lifescan Inc., Milpitas, CA, USA). Body weight and backfat thickness at P_2 (Renco Lean-Meter, Renco Corporation, Minneapolis, MN, USA) were measured in all animals at d 0 (onset of second estrus), d 7, d 15 and at the onset of the third estrus.

Of the 55 gilts initially allocated to treatment, one (HR+I) had polycystic ovaries at slaughter, five (four RH and one HR+I) had fertilization rates around 10%, and five (two RH, one HR and two HR+I) had embryos with more than two cells at collection. Data from these animals were excluded from the final analysis of treatment effects on oocyte developmental competence, which was therefore based on 13 RH, 18 HR and 15 HR+I gilts. In a sub-group of 24 animals designated to receive the jugular catheters, one gilt (RH) became sick during the experimental period, and therefore metabolic and endocrine status were established in 23 animals (seven RH, eight HR and eight HR+I). If it was not possible to allocate three littermates to treatments, pairs of gilts were randomly allocated to the HR and HR+I main treatments and matched with a non-littermate RH gilt according to body weight and age at puberty. However, of a total of 19 groups, only three (one of which was in the sub-group of canulated gilts) were matched with a non-littermate gilt.

All experimental procedures were carried out in accordance with the guidelines of the Canadian Council for Animal Care and under authorization from the Faculty Animal Policy and Welfare Committee of the University of Alberta.

5.2.2 Insemination and embryo recovery

Starting at d 18, gilts were checked for estrus every 6 h (0600, 1200, 1800 and 2400) using the back pressure test during periods of fence line contact with mature vasectomized boars. Gilts were artificially inseminated 12 and 24 h after the first observed standing estrus with pooled semen (3 x 10^9 spermatozoa/dose) from the same group of boars (Alberta Swine Genetics Corporation, Leduc, AB, Canada) specifically designated for this experiment. Time of ovulation was monitored using transcutaneous ultrasonography (Pie Medical Scanner 200, model 41480, Can Medical, Kingston, Ontario, Canada), using a 5.0–7.5 MHz multiple angle transducer to scan for the presence of pre-ovulatory follicles. Gilts were scanned every 6 h, beginning 24 h after onset of standing estrus, until completion of ovulation. During scanning, follicle diameter was recorded. Time of ovulation was defined as the time of the first scanning when no presumptive ovulatory follicles were seen less 3 h. In the group of gilts that did not have jugular catheters, 2-mL blood samples were taken by acute venipuncture at ovulation, 12 h after ovulation, 48 h after onset of standing heat and at surgery for progesterone assay. In the 48 h sample after onset of standing heat, as heat detection was performed at 6-h intervals, the timing was adjusted so that it could be exactly the same time point as in the first experiment, when heat detection was performed at 12-h intervals.

Surgeries were performed under general anesthesia 12 to 20 h after predicted time of ovulation to recover early-fertilized oocytes. The surgical procedure included laparotomy and exposure of the uterine horns, oviducts and ovaries. Ovulation rates were recorded and each oviduct was flushed twice using 5 mL of Dulbecco's Phosphate Buffered Saline (DPBS, Sigma Chemical Company, St. Louis, MO, USA), previously warmed at 39° C. Flushings were collected in sterile Falcon dishes (Fisher Scientific, St. Louis, MO, USA) and immediately transported to the *in vitro* laboratory in a Styrofoam box containing a tray and flasks filled with warm water to avoid cooling of the recovered oocytes.

Falcon dishes containing 2 mL of NCSU-23 culture media supplemented with 4 mg/mL BSA (Sigma Chemical Co., St. Louis, MO, USA, catalogue # 8022) were prepared and left in incubators to warm and pre-gas for at least 2 h before surgery. Embryos were immediately transferred into the culture dishes and incubated under standard conditions of 39° C and 5% CO_2. Embryo development was observed three times a day (0800, 1400, 2200) for seven days using a dissecting microscope

(Wild M3, Wild Heerbrugg, Switzerland) and 16X and 40X magnification to determine stage of development. Indication of fertilization (presence of sperm heads in the zona pellucida) and abnormal features (cells dividing unevenly) were observed under an inverted stage, phase-contrast, microscope at 400X magnification (Nikon Corp., Tokyo, Japan).

5.2.3 Blood sampling

At d 13 of the treatment cycle, an indwelling jugular catheter was surgically implanted via the superficial cephalic vein (Cosgrove et al., 1993) in a sub-set of 8 gilts per treatment. Three-mL blood samples were withdrawn at 15-min intervals from 0600 to 1800 on d 15 and 16 of the estrous cycle for analysis of LH. Additional 10-mL samples were collected hourly for analysis of FSH, estradiol, progesterone, insulin, IGF-I, leptin, and free and total triiodothyronine (T3) by RIA. Starting at d 18, 10-mL samples were collected every 6 h (0600, 1200, 1800 and 2400) until surgery for analysis of the same parameters. Blood samples were collected into heparinized tubes, centrifuged at 1,500 x g for 15 min, and the plasma was then decanted and stored at –30° C until analysis.

5.2.4 Estimation of plasma hormone concentrations

For RIA analysis, all treatment groups were represented in each assay, and all samples from a gilt were analyzed in the same assay. The sensitivity of the assay was calculated using the following equation: average of the zero binding tube (Bmax) – 2SD(Bmax)/average(Bmax) * 100. Plasma progesterone concentrations were determined in duplicate using an established radioimmunoassay (Coat-a-Count Progesterone, Diagnostic Products Corporation, Los Angeles, CA, USA), previously validated for use with porcine plasma without extraction (Mao and Foxcroft, 1998). The sensitivity of the assay was 0.1 ng/mL. The intra- and inter-assay CV were 7.0% and 8.3%, respectively.

Plasma LH and FSH concentrations were determined in duplicate using the homologous double antibody radioimmunoassays previously described by Cosgrove et al. (1991). For LH, 200 μL of plasma was assayed, the intra- and inter-assay CV were 15.7% and 10.4%, respectively, and the sensitivity of the assay was 0.03 ng/mL. For FSH, 300 μL of plasma was assayed, the intra-assay CV for the single assay used was 9.8%, and the sensitivity of the assay was 12.4 ng/mL.

Plasma insulin and IGF-I concentrations were determined in duplicate using the homologous double antibody radioimmunoassays previously described by Cosgrove et al. (1992). For insulin, 100 μL of plasma was assayed, the intra- and inter-assay CV were 7.0% and 12.8%, respectively, and the sensitivity of the assay was 0.02 ng/mL. For IGF-I, 100 μL of sample was initially extracted by the addition of 1 mL of diethyl ether and the radio inert recovery efficiency was 111.0%. The intra- and inter-assay CV were 6.3% and 10.5%, respectively, and sensitivity of the assay was 8.0 ng/mL.

Plasma leptin concentrations were analyzed using the multispecies double-antibody kit assay (Linco Research, St. Louis, MO, USA) previously validated in our laboratory for use with porcine plasma (Mao et al., 1999). The intra- and inter-assay CV were 15.0% and 6.3%, and the sensitivity of the assay was 1.0 ng/mL.

For determination of plasma estradiol-17β concentrations, 1 mL of plasma was extracted by the addition of 5 mL diethyl ether (VWR Canlab, Mississauga, ON, Canada) and vortexing for eight 1-minute pulses. Samples collected on d 15 and 16 of the cycle were only assayed for estradiol if gilts had low or falling levels of progesterone on these days, indicating that luteolysis had occurred and that estrogenic follicles might be developing. Therefore, estradiol analysis was performed on d 16 samples from 14 animals (all balanced for treatment and for littermate groups). Plasma samples obtained at the time of the LH surge and in the previous 24 h period were pre-diluted 2.5-fold with assay buffer (phosphate buffered saline containing 0.1g% gel, pH 7.0), while the samples collected on d 16 of the cycle and the other 6-hourly samples collected in the peri-estrus period were not diluted. Extraction tubes were placed in a liquid nitrogen/methanol bath and the aqueous layer was allowed to freeze. The solvent supernatant was poured off and dried under vacuum. Average extraction efficiency was 80 ± 0.7% and data were not corrected for recovery. Estradiol concentrations were estimated in a single radioimmunoassay using a double antibody kit from Diagnostics Products Corporation (Los Angeles, California, USA), previously validated for use with porcine plasma (Yang et al., 2000b). The intra-assay CV for the single assay used was 7.8%. The assay sensitivity was 0.3 pg/mL.

Plasma total T3 concentrations were measured using Diagnostic Products Corporation Coat-A-Count Total T3 kit (Diagnostics Product Corporation, Los Angeles, California, USA., catalogue number TKT31) with the following modifications: 1) removal of the unbound fraction of radiolabelled total T3 was done by aspiration rather than by decanting. The number of tubes aspirated was limited to 5 minutes per assay (200 tubes with two people aspirating) based on testing for drift in maximal bound tubes; 2) the volume of plasma assayed was 200 μL; 3) standards were topped up to 200 μL using zero calibrator, and 4) an extra standard was made by diluting the lowest standard provided with zero calibrator, providing a range of curve standards of 10, 20, 50, 100, 200 and 600 pg /100 μL. Parallelism was shown by finding no significant deviation between the slopes of the standard curve and a curve of volume versus binding for a pool of porcine plasma at 200, 100 and 50 μL. Recovery of 100 pg of total T3 from porcine plasma was 90 ± 7.1%. The intra- and inter-assay CV were 8.2% and 14.5%, respectively, and assay sensitivity was 11 pg/tube, equivalent to 55 pg/mL of plasma. Data were not corrected for cold recovery.

Free T3 was measured using Diagnostic Products Corporation Coat-A-Count Free T3 kit (Diagnostics Product Corporation, Los Angeles, California, USA, catalogue number TKF31) with the following modifications: 1) removal of the unbound fraction of radiolabelled free T3 was done by aspiration rather than by decanting. The number of tubes aspirated was limited to 10 minutes per assay (400 tubes with two people aspirating) based on tests for drift in maximal bound tubes; 2) the volume of plasma assayed was 200 μL; 3) standards were topped up to 200 μL using zero calibrator, and 4) an extra standard was made by diluting the lowest standard provided with zero calibrator, providing a range of curve standards of 0.025, 0.05, 0.16, 0.37, 0.75, 2.20, and 4.70 pg/100 μL. Parallelism was shown by finding no significant deviation between the slopes of the standard curve and a curve of volume versus binding for a pool of porcine plasma at 200, 100 and 50 μL. Recovery of 0.52 pg of free T3 from porcine plasma was 120.1 ± 7.2%. The intra- and inter-assay CV were 16.6% and 31.7%, respectively, and assay sensitivity was 0.02 pg/tube, equivalent to 0.1 pg/mL of plasma. Data were not corrected for cold recovery.

Analysis of both free and total T3 during the peri-estrus period were performed in the samples collected at 0600.

5.2.5 Statistical analysis

Data were analyzed as a randomized complete block design, each block consisting of three littermates representing each treatment. Treatment effects on ovulation rate, number of embryos recovered, recovery rate, fertilization rate, interval from last insemination to ovulation, estrus duration, time of ovulation in relation to estrus onset, and body weight and backfat changes were analyzed using the general linear model (GLM) procedure of the Statistical Analysis System (SAS, 1990) and based on 55 gilts (18 RH, 19 HR and 18 HR+I). The analysis of body weight and backfat changes during treatment included the effects of block and treatment in the model, with body weight and backfat at d 0 of the treatment cycle as covariates. After arc sin transformation of the data, evaluation of treatment effects on embryo developmental competence in vitro was performed by the general linear model (GLM) procedure of the Statistical Analysis System (SAS, 1990), and based on 46 gilts (two RH, one HR and two HR+I gilts were excluded from this analysis as they had embryos with more than two cells at the time of collection, and four RH and one HR+I gilts were also excluded as they had fertilization rate around 10%). For both sets of data, the complete model included treatment and block as the main effects, gilt was the experimental unit, and gilt within treatment by block interaction was used as the error term. In the event that significant treatment effects were established, multiple comparisons were performed using probability of differences (pdiff) between least square means, adjusted by Tukey-Kramer (SAS, 1990). For evaluation of treatment effects on endocrine and metabolic parameters, data were analyzed by the repeated measures analysis of variance (SAS, 1990). The variables that did not represent a normal distribution (progesterone, IGF-I, leptin, FSH) were log transformed. The complete model included treatment and block as the main effects, day as the repeated measure, gilt as the experimental unit, and gilt within treatment by block interaction was used as the error term. Analysis of plasma insulin, leptin, free and total T3 concentrations on d 15 and 16 were performed using means from two time periods: preprandial (2 h before the morning feed) and postprandial (1 to 10 h after morning feed). In the event that significant treatment effects were established, multiple comparisons were performed using probability of differences (pdiff) between least square means, adjusted by Tukey-Kramer (SAS, 1990).

Plasma LH data were characterized initially with the sliding window technique of Shaw and Foxcroft (1985), and maximum, mean, and minimum LH concentrations at d 15 and 16 were used for statistical analysis.

5.3 Results

5.3.1 Effect of litter

Litter effects on body weight, backfat, reproductive, metabolic and endocrine parameters are summarized in Table 5.1.

A positive association was observed between estrous cycle length and progesterone concentrations on d 15 (r = 0.86, P = 0.0001) and d 16 (r = 0.90, P = 0.0001). Positive correlations were also observed between estrous cycle length and body weight at third estrus (r = 0.41, P = 0.02), and between body weight at d 15 and at estrus and age at puberty (r = 0.56, P = 0.0001; r = 0.33, P = 0.06, respectively).

5.3.2 Feed intake

Data on feed intake, energy and lysine consumed are summarized in Table 5.2. RH gilts had lower (P = 0.0001) energy and lysine intakes during the period of feed restriction (d1 to d 7) compared to HR and HR+I gilts. Similarly, energy and lysine intakes were lower (P = 0.0001) in HR and HR+I gilts during the period of feed restriction (d 8 to d 15) compared to their RH counterparts. Following d 15, no differences in energy or lysine intakes were observed among groups ($P > 0.05$).

5.3.3 Body weight and backfat changes

Data on body weight and body weight change, backfat thickness at P_2 and backfat change of gilts are summarized in Table 5.3.

Feed intakes recorded, relative to changing metabolic body weight, produced expected differences in growth (body weight change). RH gilts had a lower growth rate ($P = 0.09$) during the period of feed restriction (d 1 to d 7) compared to their HR and HR+I counterparts. Moreover, HR gilts also had a lower growth rate ($P = 0.02$) during this period of feed restriction (d 8 to d 15) compared to RH and HR+I. There was no difference ($P > 0.05$) in growth rate among groups from d 16 until onset of third estrus, but feed intake was relatively low during this period. Backfat changes were not different among groups during the experimental period.

5.3.4 Reproductive characteristics

Reproductive characteristics of gilts (Table 5.4) showed that ovulation rate increased ($P = 0.06$) in the insulin treated group compared to RH, but was similar to the HR group. Consequently, the number of embryos recovered was higher ($P = 0.056$) in HR+I compared to RH, and was similar to the number in HR gilts. However, previous nutrition and insulin treatments did not affect embryo recovery rate or fertilization rate.

In the embryo data set, recovery rate was higher ($P = 0.06$) in HR+I compared to RH, and was similar to HR gilts.

5.3.5 Embryo developmental competence

The development of embryos recovered from each treatment group is summarized in Figure 5.2. There was a gradual decrease in the percentage of embryos reaching successive stages of early development and a marked decrease in the percentage of embryos making the transition from morula to blastocyst. No treatment effects were observed on the development of embryos to the 4-8 cell and morula stages, nor on the percentage of blastocysts obtained at 96 and at 144 h of culture.

5.3.6 Estrous cycle length, estrus duration, insemination and time of ovulation

Previous nutritional regimen did not influence cycle length and duration of estrus among groups (Table 5.4). A significant association was established between estrous cycle length and body weight at the onset of third estrus ($r = 0.41$, $P = 0.02$). Treatment affected time of ovulation relative to the onset of estrus, which occurred later ($P = 0.02$) in the HR+I gilts compared to their RH and HR counterparts. However, in the data set of the canulated gilts, ovulation occurred earlier ($P = 0.024$) in RH gilts compared to their HR and HR+I counterparts. There was no treatment effect on the interval from last insemination to ovulation.

5.3.7 Glucose measurements

Blood glucose concentrations were lower ($P = 0.0001$) in the HR+I gilts on d 8, 12 and 15 compared to RH and HR gilts, which had similar glucose concentrations (Table 5.5).

5.3.8 Plasma hormone concentrations (Table 5.6)

The hormone profiles of three littermate gilts are shown in Appendix 5.3 to 5.12.

Plasma insulin concentrations increased after the morning feeding on d 15 ($P = 0.0001$) and d 16 ($P = 0.0001$), and a time by treatment interaction ($P = 0.009$) on d 15 resulted in higher postprandial insulin concentrations in the HR+I group compared to the RH and HR groups. There was no time by treatment interaction on d 16. The difference between pre- and postprandial insulin was influenced by the interaction between day and treatment ($P = 0.008$), with higher increases occurring in the HR+I compared to RH and HR on d 15, and no differences on d 16. However, there was no overall effect of interaction between day and treatment in preprandial mean plasma insulin concentrations. Treatment did not affect mean plasma insulin concentrations during the peri-estrus period (Table 5.8).

Plasma IGF-I concentrations did not change after the morning feeding ($P > 0.05$). However, there was a day effect ($P = 0.055$) for mean plasma IGF-I concentrations, which increased from d 15 to d 16. Although much of this effect appears to relate to changes in HR gilts (Table 5.6; analysis based on log transformed data), no overall day by treatment interaction was observed. Treatment did not affect mean plasma IGF-I concentrations during the peri-estrus period (Table 5.8).

Plasma leptin concentrations increased after the morning feeding on d 15 ($P = 0.0001$) and on d 16 ($P = 0.055$). There were no overall effects of day, treatment or interaction between day and treatment, on plasma leptin. Also, the difference between pre- and postprandial leptin was not influenced by day or treatment. In the peri-estrus period, a trend for a time by treatment interaction was observed ($P = 0.08$); RH gilts had higher ($P = 0.02$) leptin concentrations at the time of the LH surge (time 0) compared to HR and HR+I gilts (Figure 5.3). Significant associations were established between leptin concentrations at the time of the LH surge and backfat measurements at d 15 ($r = 0.56$, $P = 0.02$) and at the onset of third estrus ($r = 0.53$, $P = 0.03$).

Plasma concentrations of total T3 increased after the morning feeding on d 15 ($P = 0.0001$) and on d 16 ($P = 0.025$). There was a significant time by treatment interaction ($P = 0.009$) on d 15, resulting in higher postprandial concentration in the HR group compared to HR+I, but similar to RH. There was a trend ($P = 0.07$) for a time by treatment interaction on d 16. The difference between pre- and postprandial total T3 was influenced by day ($P = 0.004$) and by treatment ($P = 0.009$), with higher increase occurring in the HR compared to HR+I, but similar to RH gilts on d 15 and 16 (Table 5.5). Similarly, plasma concentrations of free T3 increased after the morning feeding on d 15 ($P = 0.0001$) and on d 16 ($P = 0.009$). However, no time by treatment interaction was observed. The difference between pre- and postprandial free T3 was influenced by day ($P = 0.013$), as it decreased on d 16. However, no day by treatment interaction was observed. Preprandial mean plasma free and total T3 concentrations increased from d 15 to d 16 in all treatment groups ($P = 0.008$ and $P = 0.004$, for free and total T3, respectively; Table 5.6). However, no overall interaction between day and treatment was observed. There was no effect of treatment on postprandial mean plasma free and total T3 among groups. During the peri-estrus period, plasma free and total T3 concentrations were not affected by previous nutritional treatments (Table 5.8).

Mean and minimum LH concentrations increased ($P = 0.045$, and $P = 0.001$, respectively; Table 5.7) from d 15 to d 16; however, no treatment by day interaction was observed ($P > 0.05$). Maximum LH concentrations did not change from d 15 to 16 and no treatment by day interaction was observed (Table 5.7). During the peri-estrus period, a time by treatment interaction ($P = 0.03$) was observed and the area under the LH peak was higher ($P = 0.048$) in the RH gilts compared to HR gilts, but was similar to HR+I gilts (Figure 5.4).

Plasma FSH concentrations were not affected by day. However, there was a trend for a day by treatment interaction ($P = 0.07$; Table 5.7). During the peri-estrus period, there was a significant time by treatment interaction ($P = 0.04$) and at the time of the LH surge (time 0), FSH concentrations in HR gilts were lower ($P = 0.06$) compared to their RH and HR+I counterparts (Figure 5.4).

Progesterone concentrations decreased from d 15 to d 16 ($P = 0.0001$); however, there was no treatment by day interaction ($P > 0.05$). In the peri-ovulatory period, a treatment by time interaction was observed ($P = 0.049$; Figure 5.4). A comparison of slopes fitted to the rising progesterone concentrations showed that previous nutrition and insulin treatment affected the pattern of the progesterone rise after the LH surge ($P = 0.036$), with a slower rise in progesterone in the HR gilts compared to their RH and HR+I counterparts. However, progesterone concentrations at ovulation, 12 h after ovulation, 48 h after onset of estrus and at surgery were not different (Table 5.9). A significant correlation was established between ovulation rate and progesterone concentrations at surgery ($r = 0.45$, $P = 0.004$).

There was no effect of previous nutritional treatment on plasma estradiol concentrations at d 16 ($P > 0.05$). During the peri-estrus period, RH gilts had higher

(P = 0.01) estradiol concentrations, as shown by the area under the estradiol peak, compared to HR gilts, but had concentrations similar to HR+I gilts (Figure 5.4).

The intervals between the estradiol peak and the LH peak, LH peak and ovulation, and between the onset of estrus and either the estradiol peak or the LH peak, were not affected by previous nutritional treatments (Table 5.10).

5.4 Discussion

The effect of litter on the parameters shown in Table 5.1 revealed the importance of the use of littermates when designing experiments of this kind, and the ability to adjust for this family effect in the statistical analysis by using a randomized complete block design, fitting litter as a block to account for the differences due to family. An interesting finding of our study was the litter effect on estrous cycle length. Our results showed that cycle length was apparently determined by the length of the luteal phase, as cycle length was highly and positively associated with plasma progesterone concentrations on d 15 and d 16. Although the hormonal events of the peri-estrus period did not vary with litter, data in Figure 5.1 suggest that the interval between luteolysis and the rise in plasma estradiol was probably shorter in short-cycle gilts. However, incomplete data on the time of the initial fall in plasma progesterone did not allow us to test this relationship statistically across all litters. What determined this early onset of luteolysis could not be defined; however, timing of prostaglandin release from the uterus, the increase in number of $PGF_{2\alpha}$ receptors, and the activation of post-receptor mediators (i.e. phosphoinositol metabolism, protein kinase C), which may enhance the sensitivity of the corpus luteum to prostaglandin may play a role (Estill et al., 1993). Notwithstanding the litter effect on estrous cycle length, the positive correlation between body weight at third estrus and cycle length observed suggests that, besides litter, body weight may also play a role in determining length of the estrous cycle (heavier gilts have longer cycles). However, as body weight at d 16 and at third estrus were positively correlated with age at puberty, the influence of body weight on cycle length may be a consequence of the age at which the gilts started cycling (i.e. gilts that reached puberty later have higher body weights at third estrus). However, in the limited data set available from this study, age at puberty was not significantly correlated to length of the estrous cycle. Although no litter effect was observed for ovulation rate in the present study, in contrast to our previous studies (Almeida et al., 2000), litter may be an important factor influencing ovulation rate.

Consistent with the data reported by Almeida et al. (2000), the patterns of feed intake imposed during the estrous cycle, and the recorded differences in energy and lysine intake relative to metabolic body weight (Table 5.2), resulted in differential patterns of body weight change in RH, HR and HR+I gilts. During the period of feed restriction (d 1 to 7), gilts in the RH treatment group gained less than their counterparts fed a high plane of nutrition (HR and HR+I). Additionally, when feed intake was adjusted for metabolic body weight on d 7, HR gilts also had a lower growth rate during their period of feed restriction (d 8 to 15) compared to RH gilts. Overall, these findings confirm the observations of Williams et al. (1974) that food availability is the most important environmental factor that can affect growth. Furthermore, Booth et al. (1994) reported that gut fill clearly plays a role in live weight variation and would tend to mask initial effects of feed restriction and exaggerate effects of an increase in feed intake. The absence of an effect on backfat change is likely due to the modest level of feed restriction used (2.1 versus 2.8 X maintenance) which allowed the animals to continue growing, even during periods of restricted feeding. The lack of an effect of feed restriction on d 8 to 15 in HR+I gilts is interesting. Several studies in humans suggested that hyperinsulinemia leads to an increase in body weight (Odeleye et al., 1997; Purnell and Brunzell, 1998; Guven et al., 1999). Hyperinsulinemia is proposed to play a role in weight gain through its lipogenic actions. In response to mixed meals, insulin promotes fat storage and causes preferential oxidation of carbohydrate over fat. Furthermore, insulin increases lipoprotein lipase activity in the adipose tissue but decreases its activity in skeletal muscle, thus decreasing fat oxidation in the muscle and increasing its storage in the adipose tissue (Eckel et al., 1995). However, as there was no effect of treatment on backfat, insulin apparently increased protein deposition in feed restricted gilts.

Insulin treatment resulted in a significant increase in plasma postprandial insulin concentrations on d 15 in the HR+I group, as anticipated. This elevation of insulin was accompanied by a decrease in glucose concentrations, without any effect on plasma IGF-I, or pre- and postprandial leptin. Several studies have reported reduced levels of insulin and IGF-I with feed restriction in pigs (Booth et al., 1994; Zak et al., 1997a; Quesnel et al., 1998) and sheep (Miller et al., 1998; Snyder et al., 1999), as well as reduced levels of postprandial leptin (Mao et al., 1999; Barb, 1999). In the present study, the lack of an effect of feed restriction on plasma concentrations of these metabolic hormones on d 15, and similar plasma insulin and blood glucose concentrations in RH and HR gilts, is likely due to the modest level of feed restriction used (2.1 versus 2.8 X maintenance). On d 16, when all animals were fed a high plane of nutrition and no insulin injections were given, plasma insulin concentrations were similar among groups.

The increase in plasma concentrations of total and free T3 after feeding in the present study is in agreement with the findings of Dauncey et al. (1983) in pigs and Williams et al. (1996) in primates. Collectively, these data suggest that a meal directly stimulates the secretion of T3 from the thyroid gland, as well as affecting peripheral conversion of T4 to T3, which has been shown to be influenced by metabolic substrate availability (Mariash and Oppenheimer, 1985). Notwithstanding the increase in thyroid hormones observed after feeding, exogenous insulin injections affected the postprandial rise in total T3, which was lower in HR+I gilts compared to their HR counterparts on d 15. A similar trend was observed in postprandial free T3. If thyroid hormones are regulators of energy homeostasis, low glucose concentrations in HR+I gilts may have acted as a sign of energy deficiency, which would explain the low plasma T3 concentrations in these animals on d 15. These findings are in agreement with studies in pigs (Booth et al. 1994) and horses (Christensen et al., 1997), in which feed deprivation reduced plasma concentrations of T3 and refeeding rapidly increased them. Moreover, on d 16 when insulin injections were not given, postprandial increases in T3 were similar among treatment groups.

It has been demonstrated in gilts that the principal effect of feed restriction is to compromise the GnRH pulse generator (Armstrong and Britt, 1987), and realimentation or glucose infusion restores LH pulses (Booth, 1990), while insulin restores follicular growth (Britt et al., 1988). In feed-restricted lactating sows, glucose infusion did not affect pulsatile LH secretion (Tokach et al., 1992), although reduced LH secretion in severely catabolic sows was associated with lower postprandial insulin (Zak et al., 1997a). Additionally, glucose administration in ewes during the luteal phase of the estrous cycle did not alter LH secretion (Downing et al., 1995; Rubio et al., 1997). These results suggest that metabolic state can affect the LH response to a stimulus such as glucose. Furthermore, increases in ovulation rate were reported in cyclic gilts subjected to "flush feeding", and associated with an increase in LH pulse frequency and plasma FSH and insulin (Cox et al., 1987; Flowers et al., 1989; Beltranena et al., 1991). However, in one experiment in the study of Cox et al. (1987), increases in ovulation rate stimulated by dietary energy or exogenous insulin were not accompanied by increased LH. An essential role for central effects on LH secretion as a component of responses to changes in nutrition and metabolic state was further studied in ovariectomized gilts with insulin depleted by diabetes mellitus and without insulin therapy for four days, and pulsatile LH secretion was not altered (Angell et al., 1996). Moreover, when ovary-intact diabetic gilts were depleted of insulin on d 12 of the estrous cycle, LH pulse frequency increased between d 12 and 18 (Cox et al., 1994). In spite of the absence of adverse effects on LH secretion, reported in these studies, follicle growth decreased and atresia increased (Matamoros et al., 1990, 1991; Meurer et al., 1991; Cox et al., 1994). Similarly, in progestagen-treated prepubertal gilts, 5 days of realimentation affected follicular development independent of changes in LH secretion (Cosgrove et al., 1992). Even if a central effect of changes in feed intake is present, the findings of Williams et al. (1996) in male monkeys suggest that insulin does not mediate the changes that occur, and suggested that fuel availability may play a key role in mediating nutrition-induced changes in the central neural drive to the reproductive axis.

In the present study, LH maximum, mean and minimum concentrations on d 15 and 16 were not affected by previous nutritional treatments. The increases in mean and minimum LH concentrations on d 16 coincided with a decrease in plasma progesterone concentrations due to luteolysis. Therefore, it is likely that progesterone exerts such a dominant effect on LH secretion that the level of feed restriction applied between d 8 and 15 of the cycle does not further modify the pattern of LH secretion. Taken together, these results suggest that LH secretion is not essential, but may be an important factor in the connection between nutrition and reproduction. However, insulin treatment may have exerted some lasting effects on ovarian function, which was evident in the peri-estrus period.

In fact, estradiol concentrations in the peri-estrus period suggest that the deleterious effects of nutritionally-induced changes in metabolic state during the luteal phase are mediated by local ovarian mechanisms that affect follicular maturation. Higher estradiol peaks in HR+I compared to HR gilts provide evidence that insulin acts at the ovarian level stimulating steroidogenesis. A less robust estradiol peak in the HR gilts may be responsible for the less robust pre-ovulatory LH and FSH surges, as increased estradiol concentrations at one point are stimulatory, rather than inhibitory, and essential for the pre-ovulatory LH surge. The more robust pre-ovulatory LH surge in RH gilts was likely responsible for the shorter time to ovulation in relation to estrus onset in these gilts compared to their HR and HR+I counterparts, as shown in the data from canulated gilts, even though there was no treatment effect on the interval from the LH surge to ovulation. Moreover, the rate of rise in progesterone after ovulation, which was similar for RH and HR+I gilts but lower for HR gilts, is in agreement with our previous observation of a delayed rise in progesterone in the HR gilts compared to RH gilts (Almeida et al., 2000), and emphasizes the role of insulin in stimulating steroidogenesis (Channing et al., 1976; Barbieri et al., 1983). Indeed, a study conducted in bovine (M. Less, unpublished data) reported that a higher, and more protracted preovulatory LH surge was associated with a greater rise in plasma progesterone, probably due to a greater steroidogenic capacity of the luteinizing granulosa cells. Taken together, these results demonstrate that the differences in hormone profiles between HR and HR+I gilts during the peri-estrus period are likely due to insulin actions at the ovarian level. Furthermore, the overall deleterious effects of feed restriction were located at the ovarian level. Even the relatively modest decrease in amino acids and energy intake (metabolic fuels) during feed restriction during the late luteal phase shown in Table 5.2 appears to have affected ovarian function, possibly by limiting the availability of intraovarian growth factors. Indeed, there are at least three growth factors that may alter gonadotropin-induced progesterone secretion in pigs: IGF-I (Urban et al., 1990), fibroblast growth factor (FGF; Biswas et al., 1988) and transforming growth factor β (TGFβ; Kubota et al., 1994). Previous studies in gilts have shown that feed restriction could reduce IGF-I concentrations (Charlton et al., 1993), whereas exogenous insulin treatment increased IGF-I concentrations in pig follicles (Matamoros et al., 1991).

Previous studies have demonstrated the presence of thyroid hormone receptors in human (Wakim et al., 1993) and porcine (Wakim et al., 1987) granulosa cells, synergism between thyroid hormones and FSH in stimulatory effects on porcine granulosa cells (Maruo et al., 1987), stimulatory effects of T3 on *in vitro* steroidogenic capabilities of porcine granulosa cells (Kirkwood et al., 1992), and hypothyroidism effects on progesterone secretion and fertility in superovulated cows (Bernal et al., 1999). However, based on the lack of treatment effects on pre and postprandial total and free T3, T3 did not appear to play a role in mediating metabolic effects on reproductive function in the present study, nor did the other analyzed metabolic regulators (IGF-I and leptin). In fact, higher leptin concentrations in RH gilts at the time of the LH surge may be due to their body fat content during that period, as plasma leptin concentrations in the peri-estrus period was positively associated with backfat at d 15 and at the onset of third estrus.

As previously mentioned, insulin effects on the ovary include decreased follicle atresia, which allows more follicles to enter the preovulatory pool, thus increasing ovulation rate (see Cox, 1997 for review). In the present study, exogenous insulin injection during the luteal phase of the cycle also increased ovulation rate.

This finding is in agreement with other studies in cyclic gilts (Cox et al., 1987), and in lactating sows (Whitley et al., 1998a). Furthermore, Ramirez et al. (1997) reported an increase in litter size and farrowing rate in sows receiving insulin injections after weaning. In contrast, Kirkwood and Thacker (1991), Quesnel and Prunier (1998) and Whitley et al. (1998b) did not find any effects on ovulation rate in lactating sows after insulin administration. Additionally, Downing and Scaramuzzi (1997) working with sheep reported no effects on ovulation rate after insulin infusion. Interestingly, a positive association between ovulation rate and progesterone concentrations at surgery indicates that both the capacity of the corpus luteum to synthesize progesterone (J. Mao, unpublished data) and the number of corpora lutea play a role in the total amount of progesterone produced in the periovulatory period. Of the other reproductive characteristics analyzed, a greater number of embryos recovered in the HR+I gilts compared to RH and HR was likely due to the greater number of ovulations in this group.

In the present experiment, our findings on culture of preimplantation embryos are consistent with our preliminary study (Chapter 4) in which previous nutritional treatment did not affect embryo developmental competence within 144 h of culture. A greater developmental competence of oocytes in gilts receiving insulin might have been expected. Insulin influences granulosa cells steroidogenic activity (Channing et al., 1976; Barbieri et al. 1983), induces LH receptors in porcine granulosa cells in culture (May and Schomberg, 1981) and maturation of porcine oocytes (Tsafriri and Channing, 1975). However, previous insulin treatment also did not affect developmental competence of early fertilized oocytes. In other species, in vitro studies in hamsters (Yu and Roy, 1999) suggested that insulin effects appear to be dependent on the maturational status of ovarian cells and the dosage. Also, consistent with our data, Eppig et al. (1998) demonstrated that insulin alone did not affect competence to complete preimplantation development of mouse oocytes grown *in vitro*.

The role of peri-ovulatory progesterone as a possible mediator of nutritionally induced effects on embryonic survival has been studied in ewes and gilts

(Ashworth et al., 1989, Ashworth et al., 1991; Jindal et al., 1997). Ashworth et al. (1991) and Jindal et al. (1997) demonstrated in gilts that progesterone injections in the early stages of pregnancy could counteract a nutritionally induced increase in embryonic loss. Using an experimental model in which the level of priming progesterone, modulated by pre-ovulatory nutrition, influenced embryo survival through direct effects on the developing oocyte in the ewe (McEvoy et al., 1995a), McEvoy et al. (1995b) reported that the provision of supplementary progesterone to ewes on a high plane of feeding during the pre-ovulatory priming phase elevated plasma progesterone and enhanced subsequent ovum development. This confirms the importance of monitoring the patterns of progesterone secretion at critical times in early pregnancy (Foxcroft, 1997) and also raises the additional possibility, previously discussed by Hunter and Wiesak (1990), that differences in follicular maturation before ovulation may be reflected in subtle, but physiologically important, differences in progesterone secretion in early pregnancy.

In conclusion, our results demonstrate that a moderate level of feed restriction imposed on gilts during the late luteal phase of the cycle does not affect plasma concentrations of metabolic hormones (insulin, IGF-I, leptin, total T3 and free T3), gonadotropins (LH and FSH) nor steroid hormones (progesterone and estradiol) during this period of feed restriction, and has latent effects on endocrine events in the peri-estrus period. Effects of feed restriction appeared to be largely counteracted by insulin treatment. Although previous nutritional and insulin treatment did not impact the early developmental competence of fertilized oocytes measured *in vitro*, treatment effects (RH versus HR) on embryonic survival to d 28 seen previously may be mediated by differences in progesterone status in early pregnancy.

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Table 5.1 Litter effects on all parameters analyzed during experimental treatment of gilts (P values)

Parameter	Litter	Day x Litter	Time x Litter
Body weight			
d 0	0.0001	-	-
d 7	0.0001	-	-
d 15	0.0001	-	-
Estrus	0.025	-	-
Growth rate			
d 1 – d 7	0.02	-	-
d 8 – d 15	0.02	-	-
Backfat			
d 0	0.02	-	-
d 7	0.09	-	-
d 15	0.001	-	-
estrus	0.003	-	-
Estrous cycle length	0.025	-	-
Estrus duration	0.08	-	-
Ovulation rate	NS	-	-
Blastocysts at 96 h	0.08	-	-
Blood glucose	-	0.052	-
Postprandial insulin	-	0.037	-
Preprandila Total T3	-	0.024	-
Postprandial Total T3	-	0.001	-
Postprandial Free T3	-	0.023	-
Preprandial leptin	-	0.044	-
Postprandial leptin	-	0.06	-
Mean LH	-	0.03	-
Minimum LH	-	0.04	-
Maximum LH	-	0.07	-
Progesterone	-	.008	-
Peri-estrus period			
Insulin	-	-	0.0004
Leptin	-	-	0.0004
IGF-I	-	-	0.0001
Total T3	0.06	-	-
Free T3	NS	-	-
Estradiol	0.001	-	-
Progesterone	-	-	0.0001
LH	NS	-	-
FSH	-	-	0.0001

Table 5.2 Daily feed consumed (g), energy intake (kcal) and lysine intake (mg) of gilts on treatment relative to metabolic body weight ($BW^{.75}$)

Parameter	RH[1]	HR[1]	HR+I[1]
Feed offered, kg			
d 1-d 7	2.8 ± 0.1	3.7 ± 0.1	3.7 ± 0.1
d 8-d 15	3.8± 0.05	2.8 ± 0.05	2.9 ± 0.05
d 16-estrus	4.0 ± 0.1	3.9 ± 0.1	4.0 ± 0.1
Feed consumed, g/kg $BW^{.75}$			
d 1-d 7	61.8 ± 1.8^{a}	73.8 ± 1.8^{b}	73.6 ± 1.8^{b}
d 8-d 15	84.8 ± 1.2^{x}	65.7 ± 1.1^{y}	68.7 ± 1.1^{y}
d 16-estrus	64.6 ± 3.1	65.4 ± 3.1	69.7 ± 3.3
Energy intake, kcal/kgBW $^{.75}$			
d 1-d 7	205.0 ± 6.2^{a}	244.5 ± 6.0^{b}	244.0 ± 6.0^{b}
d 8-d 15	281.0 ± 3.9^{x}	217.7 ± 3.7^{y}	227.7 ± 3.7^{x}
d 16-estrus	213.8 ± 9.0	225.7 ± 9.5	230.7 ± 9.5
Lysine intake, mg/kgBW $^{.75}$			
d 1-d 7	0.61 ± 0.02^{a}	0.73 ± 0.02^{b}	0.73 ± 0.02^{b}
d 8-d 15	0.84 ± 0.01^{x}	0.65 ± 0.01^{y}	0.68 ± 0.01^{y}
d 16-estrus	0.64 ± 0.03	0.65 ± 0.03	0.69 ± 0.03

[1] RH: gilts restricted (2.1 X maintenance) from d 1 to d 7, then fed a high plane (2.8 X maintenance) from d 8 until onset of estrus; HR: gilts fed a high plane (2.8 X maintenance) from d 1 to d 7, restricted (2.1 X maintenance) from d 8 to d 15 and then fed high plane again from d 16 until onset of estrus; HR+I: gilts fed as HR gilts but given insulin from d 8 to d 15.

[a,b] LS means within a row with different superscripts differ ($P < 0.0005$); [x,y] LS means within a row with different superscripts differ ($P = 0.0001$).

Table 5.3 Body weight, body weight change, backfat thickness and backfat change of RH, HR and HR+I gilts at d 0, d 7, d 15 and at estrus (LSM ± SEM)

Parameter	RH[1] (n=18)	HR[1] (n=19)	HR+I[1] (n=18)
Body weight, kg			
d 0	143.0 ± 3.0	138.5 ± 2.4	136.5 ± 2.7
d 7	144.6 ± 0.9^{x}	147.2 ± 0.7^{y}	146.8 ± 0.8^{y}
d 15	151.0 ± 0.6	151.5 ± 0.6	152.8 ± 0.7
Estrus	153.4 ± 1.6^{y}	152.8 ± 1.4^{y}	157.0 ± 1.9^{x}
Body weight change, kg			
d 1-d 7	5.0 ± 0.9^{x}	7.7 ± 0.7^{y}	7.2 ± 0.7^{y}
d 8-d 15	7.3 ± 0.6^{a}	5.3 ± 0.5^{b}	7.4 ± 0.5^{a}
d 16- estrus	1.9 ± 1.2	0.7 ± 1.0	4.0 ± 1.3
Backfat, mm			
d 0	11.6 ± 0.7	11.0 ± 0.5	12.3 ± 0.6
d 7	12.6 ± 0.3	12.7 ± 0.3	12.4 ± 0.3
d 15	13.5 ± 0.4	12.8 ± 0.3	12.7 ± 0.3
Estrus	12.5 ± 0.4	12.8 ± 0.3	12.5 ± 0.4
Backfat change, mm			
d 1-d 7	0.9 ± 0.4	0.9 ± 0.4	-0.9 ± 0.4
d 8-d 15	1.0 ± 0.3	0.4 ± 0.3	-0.04 ± 0.4
d 16-estrus	0.7 ± 0.3	0.5 ± 0.3	-0.3 ± 0.4

[1] RH: gilts restricted (2.1 X maintenance) from d 1 to d 7, then fed a high plane (2.8 X maintenance) from d 8 until onset of estrus; HR: gilts fed a high plane (2.8 X maintenance) from d 1 to d 7, restricted (2.1 X maintenance) from d 8 to d 15 and then fed high plane again from d 16 until onset of estrus; HR+I: gilts fed as HR gilts but given insulin from d 8 to d 15.

^{a,b} LSMeans within rows with different superscripts differ ($P < 0.05$); ^{x,y} LS means within rows with different superscripts differ ($P < 0.1$).

Table 5.4 Reproductive characteristics of gilts on treatment (LSM ± SEM)

Parameter	RH[1]	HR[1]	HR+I[1]
All gilt data (n = 55)	18	19	18
Ovulation rate	16.0 ± 1.8^{a}	18.0 ± 1.6^{ab}	22.0 ± 1.6^{b}
Number of embryos recovered	14.0 ± 1.8^{a}	16.4 ± 1.5^{ab}	20.3 ± 1.8^{b}
Recovery rate, %	82.8 ± 3.0	89.4 ± 2.5	92.0 ± 2.8
Fertilization rate, %	95.5 ± 5.0	87.8 ± 4.3	96.2 ± 5.0
Interval last insemination to ovulation, h	12.4 ± 1.8	12.0 ± 1.7	16.4 ± 1.8
Estrous cycle length, d	20.0 ± .3	19.8 ± .3	20.0 ± .3
Estrus duration, h	51.5 ± 2.0	52.0 ± 1.7	53.8 ± 1.7
Ovulation in relation to estrus onset, h	40.7 ± 1.5^{c}	41.7 ± 1.4^{c}	46.5 ± 1.5^{d}
Embryo data set (n = 46)	13	18	15
Ovulation rate	16.0 ± 2.2	17.7 ± 2.1	21.0 ± 2.0
Number of embryos recovered	13.5 ± 2.2^{c}	16.3 ± 1.7^{cd}	20.9 ± 2.1^{d}
Recovery rate, %	82.8 ± 3.1^{c}	88.5 ± 2.5^{cd}	93.6 ± 3.0^{d}
Fertilization rate, %	96.3 ± 6.3	87.3 ± 5.0	97.0 ± 6.0
Estrus duration, h	51.8 ± 2.8	51.7 ± 2.2	56.0 ± 2.5
Ovulation in relation to estrus onset, h	41.0 ± 2.4	42.8 ± 2.0	45.8 ± 2.0
Endocrine data set (n = 23)	7	8	8
Ovulation rate	15.8 ± 2.3	18.8 ± 1.8	19.0 ± 1.6
Number of embryos recovered	13.6 ± 1.4	17.6 ± 1.0	17.5 ± 1.1
Recovery rate, %	81.6 ± 4.3	94.9 ± 3.0	93.5 ± 3.4
Fertilization rate, %	88.3 ± 12.2	82.2 ± 8.8	94.7 ± 9.7
Estrus duration, h	52.0 ± 5.0	52.0 ± 3.0	57.4 ± 2.8
Ovulation in relation to estrus onset, h	35.8 ± 3.0^{c}	45.5 ± 1.9^{d}	48.3 ± 1.9^{d}

[1] RH: gilts restricted (2.1 x maintenance) from d 1 to d 7, then fed a high plane (2.8 x maintenance) from d 8 until onset of estrus; HR: gilts fed a high plane (2.8 x maintenance) from d 1 to d 7, restricted (2.1 x maintenance) from d 8 to d 15 and then fed high plane again from d 16 until onset of estrus; HR+I: gilts fed as HR gilts but given insulin from d 8 to d 15.

^{c,d} LS means within a row with different superscripts differ ($P \le 0.05$), ^{a,b} LS means within a row with different superscripts differ ($P \le 0.06$).

Table 5.5 Blood glucose concentrations (mg/dL) of gilts on treatment (LSM ± SEM)

Treatment[1]	Blood glucose concentrations (mg/dL)		
	d 8	d 12	d 15
RH	68.6 ± 3.7^x	65.2 ± 4.0^x	64.6 ± 3.8^x
HR	62.7 ± 2.8^x	63.5 ± 3.0^x	63.0 ± 3.0^x
HR+I	39.0 ± 2.6^y	38.3 ± 4.0^y	37.2 ± 2.8^y

[1] RH: gilts restricted (2.1 X maintenance) from d 1 to d 7, then fed a high plane (2.8 X maintenance) from d 8 until onset of estrus; HR: gilts fed a high plane (2.8 X maintenance) from d 1 to d 7, restricted (2.1 X maintenance) from d 8 to d 15 and then fed high plane again from d 16 until onset of estrus; HR+I: gilts fed as HR gilts but given insulin from d 8 to d 15.

[x,y] LS means within a column with different superscripts differ (P = 0.0001).

Table 5.6 Least square means (± SEM) of plasma IGF-I, pre- and postprandial insulin, leptin, free and total T3 concentrations on d 15 and 16 (Feeding = increase in plasma concentrations after feeding; Δ = difference between post- and preprandial plasma concentrations)

Parameter	Feeding	RH (n = 7)	HR (n = 8)	HR+I (n = 8)	Day x Treatment	Day	Treatment
IGF-I, ng/mL							
d 15	NS	105 ± 6	98 ± 5	100 ± 5	NS	0.055	NS
d 16	NS	105 ± 7	106 ± 6	102 ± 6			NS
Insulin, ng/mL							
Preprandial d 15		0.7 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	NS	NS	NS
Postprandial d 15	0.0001	1.2 ± 0.2^{a}	1.0 ± 0.2^{a}	2.0 ± 0.2^{b}	0.0005	NS	0.001
Δ Insulin d 15		0.4± 0.1	0.3± 0.1	1.1± 0.1	0.0077	0.0001	0.003
Preprandial d 16		0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	-	-	NS
Postprandial d 16	0.0001	1.1 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	NS	NS	NS
Δ Insulin d 16		0.2± 0.1	0.4± 0.1	0.4± 0.1	-	-	NS
Leptin, ng/mL							
Preprandial d 15		1.6 ± 0.5	2.0 ± 0.4	2.0 ± 0.4	NS	NS	NS
Postprandial d 15	0.0001	2.5 ± 0.4	2.7 ± 0.4	2.7 ± 0.4	NS	NS	NS
Δ Leptin d 15		0.9± 0.2	0.6± 0.2	0.7± 0.2	NS	NS	NS
Preprandial d 16		2.0 ± 0.4	2.0 ± 0.3	1.8 ± 0.3	-	-	NS
Postprandial d 16	0.055	2.5 ± 0.4	2.5 ± 0.3	2.2 ± 0.3	-	-	NS
Δ Leptin d 16		0.5 ± 0.4	0.5 ± 0.3	0.3 ± 0.3	-	-	NS
Total T3, pg/mL							
Preprandial d 15		400 ± 46	380 ± 41	447 ± 41	NS	0.004	NS

Postprandial d 15	0.0001	580 ± 38^{xy}	634 ± 34^{x}	518 ± 34^{y}	NS	NS	0.094
Δ Total T3 d 15		182 ± 38^{a}	254 ± 35^{a}	72 ± 35^{b}	NS	0.0043	0.009
Preprandial d 16		443 ± 48	463 ± 44	556 ± 44	0.072	-	NS
Postprandial d 16	0.0253	555 ± 29	584 ± 26	529 ± 26	-	-	NS
Δ Total T3 d 16		113 ± 50^{cd}	122 ± 46^{c}	-27 ± 46^{d}	-	-	0.07
Free T3, pg/mL							
Preprandial d 15		0.44 ± 0.08	0.60 ± 0.07	0.52 ± 0.07	NS	0.008	NS
Postprandial d 15	0.0001	1.05 ± 0.09	1.13 ± 0.09	0.84 ± 0.09	NS	NS	NS
Δ Free T3 d 15		0.61 ± 0.11	0.53 ± 0.10	0.33 ± 0.10	NS	0.013	NS
Preprandial d 16		0.68 ± 0.13	0.73 ± 0.12	0.69 ± 0.12	-	-	NS
Postprandial d 16	0.009	1.07 ± 0.12	0.99 ± 0.10	0.82 ± 0.10	-	-	NS
Δ Free T3 d 16		0.39 ± 0.16	0.26 ± 0.14	0.12 ± 0.14	-	-	NS

[1] RH: gilts restricted (2.1 X maintenance) from d 1 to d 7, then fed a high plane (2.8 X maintenance) from d 8 until onset of estrus; HR: gilts fed a high plane (2.8 X maintenance) from d 1 to d 7, restricted (2.1 X maintenance) from d 8 to d 15 and then fed high plane again from d 16 until onset of estrus; HR+I: gilts fed as HR gilts but given insulin from d 8 to d 15.

[a,b] LSMeans within rows with different superscripts differ ($P \leq 0.01$); [x,y] LS means within rows with different superscripts differ ($P \leq 0.08$); [c,d] LSMeans within rows with different superscripts differ ($P < 0.1$).

Table 5.7 Least square means (± SEM) of plasma LH maximum, mean and minimum, FSH and progesterone concentrations on d 15 and 16 and estradiol concentrations on d 16

Parameter	RH (n = 7)	HR (n = 8)	HR+I (n = 8)	Day x Treatment	Day	Treatment
LH, ng/mL						
Maximum						
d 15	0.55 ± 0.10	0.50 ± 0.10	0.47 ± 0.10	NS	NS	NS
d 16	0.57 ± 0.10	0.50 ± 0.05	0.50 ± 0.05			
Mean						
d 15	0.32 ± 0.04	0.30 ± 0.03	0.30 ± 0.03	NS	0.045	NS
d 16	0.37 ± 0.03	0.32 ± 0.03	0.35 ± 0.03			
Minimum						
d 15	0.17 ± 0.02	0.18 ± 0.02	0.17 ± 0.02	NS	0.001	NS
d 16	0.21 ± 0.02	0.20 ± 0.02	0.23 ± 0.02			
FSH, ng/mL						
d 15	18 ± 3	21 ± 3	20 ± 3	0.07	NS	NS
d 16	23 ± 5	15 ± 4	24 ± 4	-	-	NS
Progesterone, ng/mL						
d 15	13.38 ± 1.40	10.15 ± 1.27	12.59 ± 1.27	NS	0.001	NS
d 16	7.18 ± 2.17	5.46 ± 1.97	7.20 ± 1.97	-	-	NS
Estradiol, pg/mL						
d 16	39.47 ± 7.33	35.13 ± 6.25	41.38 ± 6.25	-	-	NS

[1] RH: gilts restricted (2.1 X maintenance) from d 1 to d 7, then fed a high plane (2.8 X maintenance) from d 8 until onset of estrus; HR: gilts fed a high plane (2.8 x maintenance) from d 1 to d 7, restricted (2.1 X maintenance) from d 8 to d 15 and then fed high plane again from d 16 until onset of estrus; HR+I: gilts fed as HR gilts but given insulin from d 8 to d 15.

Table 5.8 Plasma concentrations of metabolic hormones in the peri-estrus period (LSM ± SEM)

Parameter	RH[1] (n = 7)	HR[1] (n = 8)	HR+I[1] (n = 8)
Insulin, ng/mL	0.9 ±0.05	0.8 ±0.05	0.9 ±0.05
IGF-1, ng/mL	150 ±14	138 ±14	156 ±14
Total T3, pg/mL	502 ±34	471 ±34	560 ±38
Free T3, pg/mL	0.49 ± 0.08	0.46 ±0.08	0.64 ± 0.09

[1] RH: gilts restricted (2.1 X maintenance) from d 1 to d 7, then fed a high plane (2.8 X maintenance) from d 8 until onset of estrus; HR: gilts fed a high plane (2.8 X maintenance) from d 1 to d 7, restricted (2.1 X maintenance) from d 8 to d 15 and then fed high plane again from d 16 until onset of estrus; HR+I: gilts fed as HR gilts but given insulin from d 8 to d 15.

Table 5.9 Plasma progesterone concentrations (ng/mL) at ovulation, 12 h after ovulation, 48 h after onset of estrus and at surgery (LSM ± SEM)

Time	RH[1] (n = 7)	HR[1] (n = 8)	HR+I[1] (n = 8)
Ovulation	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
12 h after ovulation	2.6 ± 0.3	2.0 ± 0.3	2.0 ± 0.3
48 h after onset of estrus	2.1 ± 0.3	1.5 ± 0.2	1.5 ± 0.2
Surgery	2.7 ± 0.3	2.4 ± 0.3	2.0 ± 0.3

[1] RH: gilts restricted (2.1 X maintenance) from d 1 to d 7, then fed a high plane (2.8 X maintenance) from d 8 until onset of estrus; HR: gilts fed a high plane (2.8 X maintenance) from d 1 to d 7, restricted (2.1 X maintenance) from d 8 to d 15 and then fed high plane again from d 16 until onset of estrus; HR+I: gilts fed as HR gilts but given insulin from d 8 to d 15.

Table 5.10 Endocrine parameters in the peri-estrus period (LSM ± SEM)

Parameter	RH[1] (n=7)	HR[1] (n=8)	HR+I[1] (n=8)
Interval LH peak to ovulation, h	28.6 ± 2.0	33.0 ± 1.8	28.2 ± 2.0
Interval onset of estrus-LH peak, h	12.5 ± 3.6	9.7 ± 3.6	13.4 ± 3.6
Interval onset of estrus-estradiol peak, h	3.4± 4.3	-3.5 ± 4.3	-1.7 ± 4.3
Interval estradiol peak-LH peak, h	16.0 ± 2.5	14.6 ± 2.5	14.0 ± 2.5

[1] RH: gilts restricted (2.1 X maintenance) from d 1 to d 7, then fed a high plane (2.8 X maintenance) from d 8 until onset of estrus; HR: gilts fed a high plane (2.8 X maintenance) from d 1 to d 7, restricted (2.1 X maintenance) from d 8 to d 15 and then fed high plane again from d 16 until onset of estrus; HR+I: gilts fed as HR gilts but given insulin from d 8 to d 15.

Figure 5.1 Gonadotropin and steroid hormone profiles during the peri-estrus period from littermate gilts with an 18-d estrous cycle (n = 3) and a 21-d estrous cycle (n = 2). Luteal regression and a decline in plasma progesterone occurred on d 14 in the 18-d cycle gilts and on d 16 in the 21-d cycle gilts.

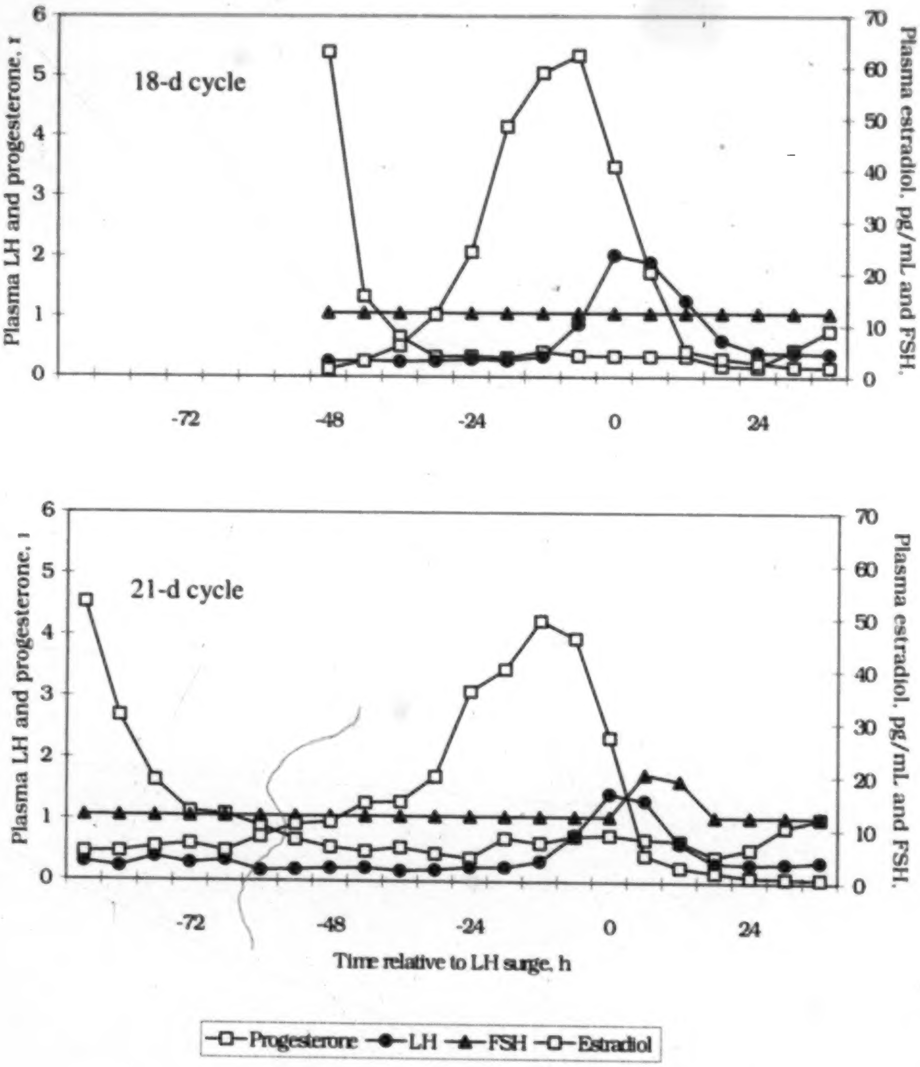

18-d cycle
21-d cycle
Plasma LH and progesterone,
Plasma estradiol, pg/mL and FSH,
0
1
2
3
4
5
6
10
20
30
40
50
60
70
-72
-48
-24
0
24
Time relative to LH surge, h
Progesterone
LH
FSH
Estradiol

Figure 5.2 Percentage of fertilized oocytes recovered 12 to 20 h after ovulation reaching successive stages of development during culture *in vitro* for 144 h.

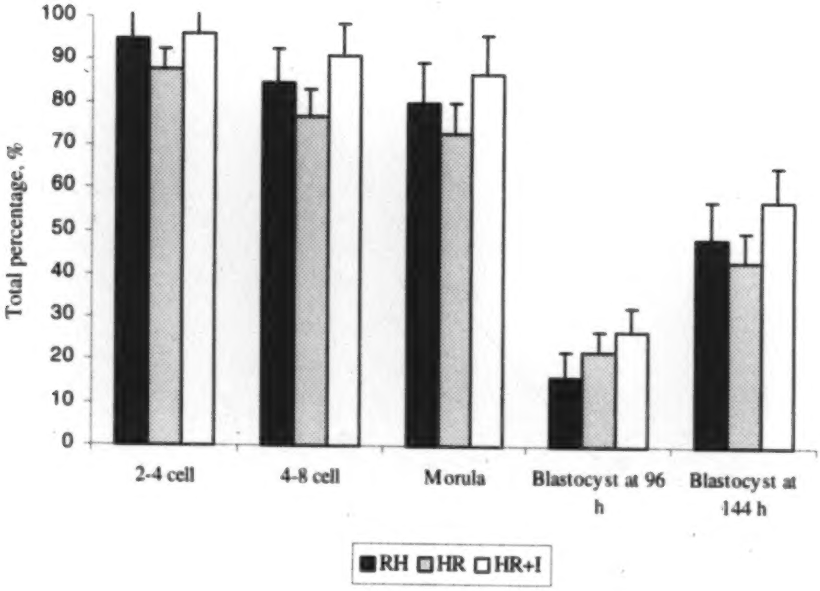

Total percentage, %
100
90
80
70
60
50
40
30
20
10
0
2-4 cell
4-8 cell
Morula
Blastocyst at 96 h
Blastocyst at 144 h
■RH
▨HR
☐HR+I

Figure 5.3 Plasma leptin concentrations during the peri-estrus period in gilts according to treatment. RH gilts have higher leptin concentrations at the time of the LH surge, compared to HR and HR+I gilts ($P < 0.05$).

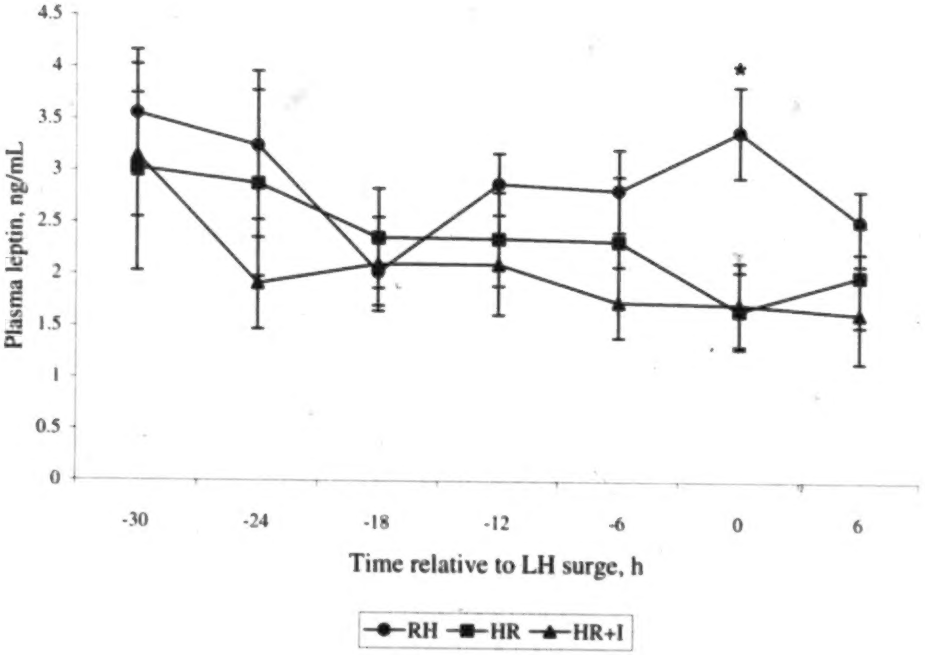
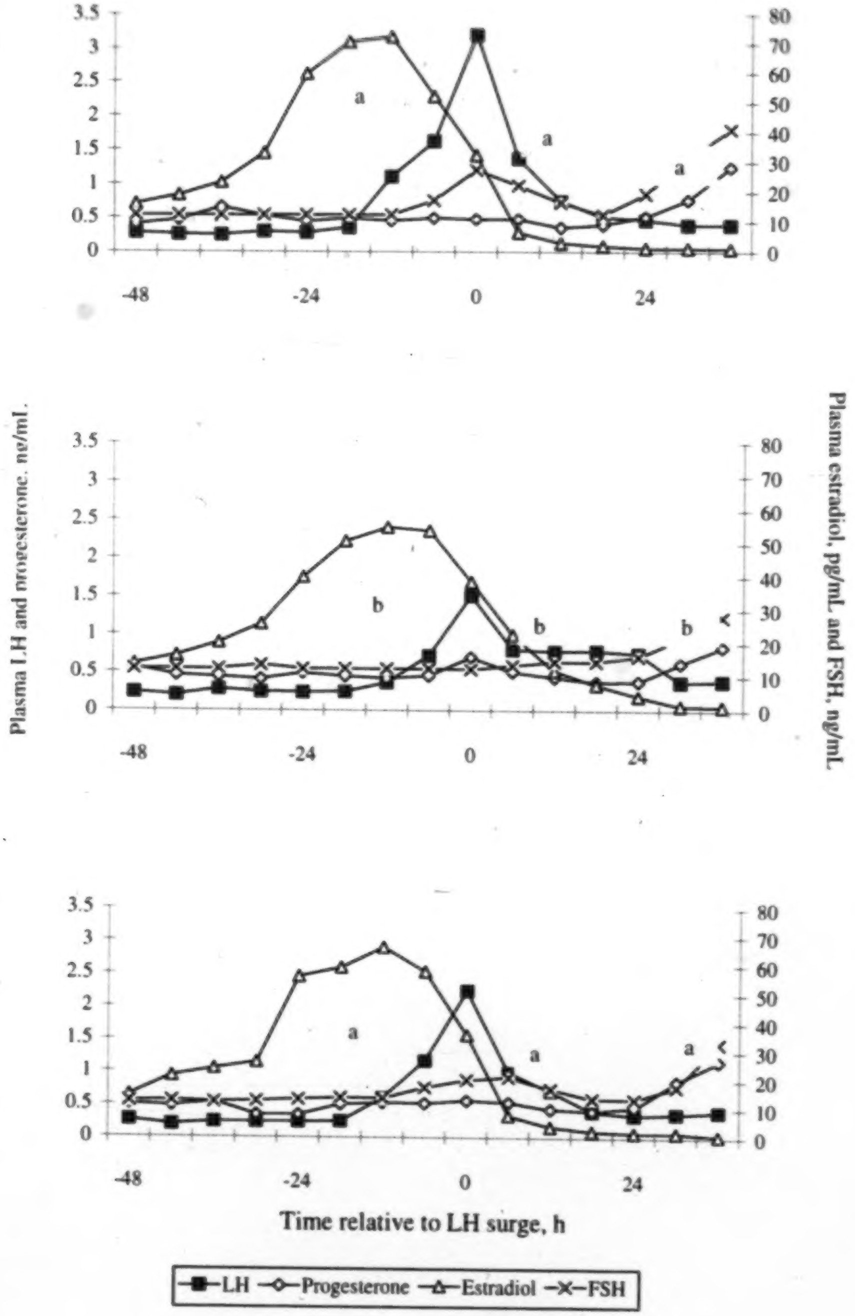

Plasma leptin, ng/mL
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
*
-30
-24
-18
-12
-6
0
6
Time relative to LH surge, h
RH
HR
HR+I

Figure 5.4 Gonadotropin and steroid profiles of gilts during the peri-estrus period according to treatment. Estradiol, LH and FSH surges are lower in HR compared to RH and HR+I gilts (a,b values differ $P < 0.05$). The progesterone rise after the LH surge occurs more slowly in HR compared to RH and HR+I gilts (a,b values differ $P < 0.05$).


Plasma LH and progesterone, ng/mL
Plasma estradiol, pg/mL and FSH, ng/mL
3.5
3
2.5
2
1.5
1
0.5
0
80
70
60
50
40
30
20
10
0
-48
-24
0
24
a
a
a
b
b
b
a
a
a
Time relative to LH surge, h
LH
Progesterone
Estradiol
FSH

CHAPTER 6

GENERAL DISCUSSION

The experimental paradigm described in the present thesis was developed from the recent study in lactating and weaned primiparous sows (Zak et al., 1997a), in which nutritional changes were used to create different patterns of catabolism during a lactation period of 28 days. In the lactating sow model, differential patterns of feed intake during lactation, and associated metabolic and endocrine changes, differentially affected the reproductive performance of the sows. Any period of feed restriction in lactation resulted in a lower ovulation rate and marginally extended weaning-to-estrus intervals compared to sows fed to appetite throughout (AA), whereas feed restriction in the last week of lactation (AR) reduced embryo survival to day 28 of pregnancy compared to both AA sows and sows restricted during the first three weeks of lactation (RA). Given the importance of gilts as a component of the breeding herd, and the need to optimize fertility in these animals, the cyclic gilt became a challenging experimental model to work with. Moreover, cyclic gilts might represent an important experimental paradigm, as their 21-day estrous cycle can be used to represent a 21-day lactation, which is becoming the norm of the swine industry. Therefore, in the gilt model developed in this thesis the aim was to use feed restriction to create comparable differences in relative metabolic state, in this case, by failing to meet the requirements of gilts for maintenance and growth by the same margins that the nutrient intake of the lactating sows failed to meet their requirements for maintenance and milk production.

Chapter 3 described the establishment of this cyclic gilt model, in which the main hypothesis tested was that different metabolic states, achieved by different patterns of feed restriction at critical times before the recruitment of follicles into the preovulatory hierarchy, would affect subsequent fertility of gilts. Chapter 4 described the use of this established gilt model to further define the mechanisms mediating nutritional effects on embryonic survival; however, the comparison was limited to the two different periods of feed restriction on the basis that this still provided the opportunity to determine effects on embryonic survival independent of effects on ovulation rate. The main hypothesis tested was that different patterns of feed intake at critical times before the recruitment of follicles into the preovulatory pool would affect follicle and oocyte maturation, leading to poor embryonic development *in vitro*. Chapter 5 described an experiment that extended the established cyclic gilt model, by investigating responses to insulin treatment during the period of feed restriction in the late luteal phase that had deleterious effects on subsequent fertility, compared to untreated gilts and gilts undergoing feed restriction in the early luteal phase. The metabolic and endocrine status of gilts during treatment and during the subsequent peri-estrus period was established. Moreover, the developmental competence of early fertilitzed oocytes *in vitro* was further investigated. The main hypothesis tested was that the deleterious effects of feed restriction and associated changes in metabolic status would be counteracted by treatment with long acting insulin. In the present chapter, the results from all experiments will be integrated in an attempt to understand the mechanisms controlling the interactions between nutrition and reproduction in the cyclic gilt. In particular, the differences between the lactating sow and the cyclic gilt experimental models merit discussion.

6.1 Relative metabolic state

The primiparous lactating sow fails to consume sufficient feed to maintain a positive energy balance (Aherne et al., 1991) and approaches an increasingly catabolic condition as lactation progresses and the sow mobilizes body tissue to maintain milk production (King and Williams, 1984; Zak et al., 1997a). On the other hand, the cyclic gilt is in the process of growth, and therefore, protein and fat are being deposited (anabolic condition) at different time points, depending upon the body weight and genetic lean growth potential of the animal (Bikker et al., 1996; Möhn and de Lange, 1998).

Perhaps surprisingly, the results in Experiment 3 indicate that the metabolic parameters measured (insulin, IGF-I, leptin, total T3 and free T3) were not affected by the level of feed restriction imposed on the gilts (2.1 versus 2.8 X maintenance).

Although backfat was not affected, a consistent result across the three experiments, a lower growth rate was evident during the different weeks of feed restriction. In contrast, lactating primiparous sows subjected to feed restriction during lactation lose more body weight and backfat, and plasma concentrations of insulin and IGF-I were significantly reduced (Zak et al., 1997a; Quesnel and Prunier, 1998; Mao et al., 1999). Both insulin (Cox, 1997) and IGF-I (Monget and Martin, 1997) have been shown to influence reproductive function.

Taken together, these observations suggest that in relation to metabolic state, the lactating sow and the cyclic gilt experimental models are not strictly comparable, as increased catabolism (lactating sow) or reduced anabolism (cyclic gilt) induce different metabolic responses. Increased catabolism in the sow is characterized by lower concentrations of insulin and IGF-I compared to animals fed ad libitum, whereas marginal growth reductions in the gilt is characterized by similar insulin, IGF-I, thyroid hormones and leptin concentrations compared to animals on a high plane of nutrition. Interestingly both models were able to cause similar deleterious effects on subsequent fertility (lower embryonic survival to day 28 of pregnancy).

6.2 Central regulation of LH release

There appear to be differences in the regulation of pulsatile release of LH in the two experimental models. In the lactating sow, the suckling stimulus provides the primary block to the resumption of estrus during lactation by reducing the pulsatile release of LH (Britt et al., 1985); however, nutritional restriction can further exaggerate this problem (Foxcroft et al., 1995; Quesnel and Prunier, 1995). Confirmation that a lack of pulsatile LH secretion is the primary cause of lactational anestrus came from studies in which treatment with exogenous GnRH during lactation resulted in follicular development, behavioural estrus and ovulation (Cox and Britt, 1982; De Rensis et al., 1991). In the cyclic gilt, the modulation of LH secretion is progesterone-dependent during the luteal phase (Cosgrove et al., 1993). Evidence for endogenous opioids acting as mediators of progesterone modulation of

LH secretion came from the study of Barb and coworkers (1986), in which the opioid antagonist naloxone was found to increase LH secretion only in the luteal phase, when the concentration of plasma progesterone was high. In Experiment 3, the increase in minimum and mean LH concentrations coincided with the decrease in plasma progesterone concentrations due to luteolysis, consistent with the concept of progesterone be a key regulator of LH secretion.

From the data obtained from gilts that were still dominated by progesterone at the time of sampling on day 15, it appears that in the cyclic gilt model established in this series of experiments, the level of feed restriction (2.1 X maintenance) to which the gilts were subjected was not severe enough to trigger changes in key metabolic hormones or to totally suppress episodic LH secretion during the cycle. However, embryonic survival to day 28 of pregnancy was still reduced in gilts restricted in the second week of the cycle, and changes in LH secretion clearly did not mediate this effect. Based on the evidence reviewed by Foxcroft (1997), differences in progesterone secretion in the immediate post-ovulatory period may mediate treatment effects on embryonic survival. The origin of these differences in progesterone may likely be due to differences in follicle maturation and a working hypothesis of the cascade of events linking previous nutritional treatment to subsequent differences in embryonic survival is summarized in Figure 6.1. The level of feed restriction imposed on the gilts in the late luteal phase (2.1 X maintenance), before the recruitment and selection of follicles into the pre-ovulatory pool, alters the intra-ovarian metabolic milieu, thus affecting follicular maturation and the endocrine events during the peri-estrus period. Incomplete follicular maturation firstly affects the size of the pro-estrus surge in estradiol. In turn, this smaller surge of estradiol is not sufficient to trigger a robust pre-ovulatory surge in LH, which is essential for the final stages of maturation of the oocyte and for the initiation of luteinization. Finally, the less robust LH surge affects the pattern of luteinization of the follicles, giving rise to corpora lutea that produce less progesterone in the

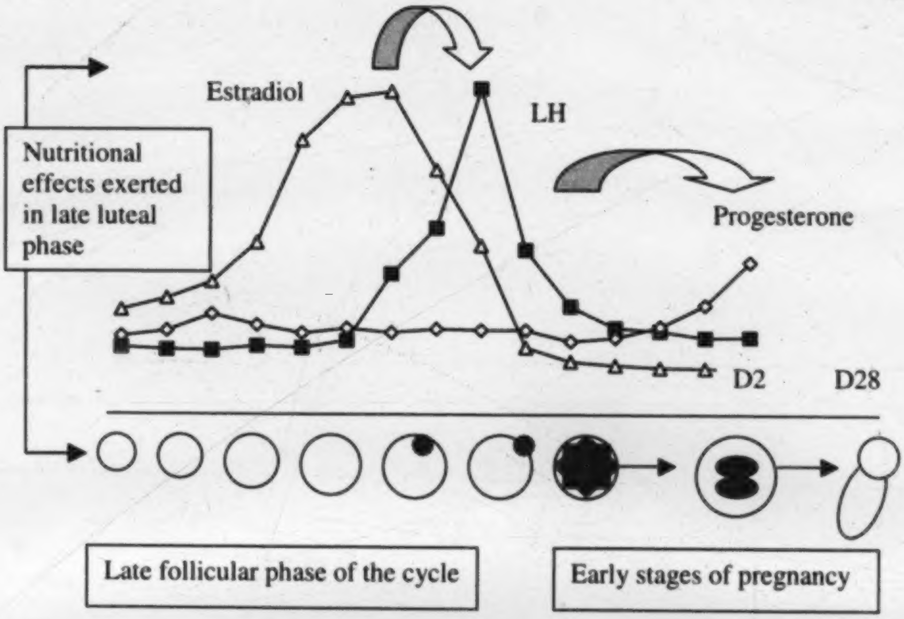

Estradiol
LH
Progesterone
Nutritional effects exerted in late luteal phase
D2
D28
Late follicular phase of the cycle
Early stages of pregnancy

Figure 6.1 Summary of the cascade of events linking previous nutritional treatment to subsequent differences in embryonic survival.

immediate post-ovulatory period. Then, as suggested by Ashworth (1991) and Jindal et al. (1997), peri-ovulatory progesterone could be the ultimate mediator of nutritionally induced effects on embryonic survival in gilts.

In contrast to the present results, other experimental paradigms using gilts have reported changes in the pattern of LH pulsatility due to feed restriction. Armstrong and Britt (1987) reported that chronic feed restriction resulted in cessation of estrous cycle in gilts due to altered secretion of GnRH from the hypothalamus. In their study, the restricted animals received 0.23 kg feed/day, while the controls received 1.81 kg feed/day. Moreover, the restricted animals lost about 25 kg of body weight and 8 mm of backfat (catabolism). Therefore, restriction of feed intake probably caused anestrus because energy intake was inadequate, leading to a suppression of the episodic release of LH. Moreover, increasing feed intake resulted in the resumption of estrous cycles, which was preceded by a significant increase in body weight, but not backfat, and a linear increase in plasma LH concentration and frequency of episodic LH release. Further studies using prepubertal gilts reported that realimentation after a 7-day period of feed restriction to maintenance requirements (about 50% of ad libitum intake) increased LH pulsatility within 6 hours and follicular development within day 7, which was not associated with changes in body weight or composition (Booth et al., 1994). Furthermore, this rapid enhancement of LH secretion in response to dietary repletion might have been mediated by observed changes in glucose and insulin status. Ovarian responses to gonadotropins may also be potentiated by observed increases in plasma glucose, insulin, and IGF-I (Booth et al., 1996).

Collectively, these findings suggest that our cyclic gilt model did not impact metabolic state (no difference in metabolic hormones), and, as a consequence, no central effects (at the hypothalamus or pituitary) were observed. As the pattern of LH secretion was affected in other gilt models, as shown in the studies of Armstrong and Britt (1987) and Booth et al. (1994), there must be a threshold of energy and nutrient requirements below which central effects are observed. This appears to be crossed somewhere between a feeding allowance of 1.0 and 2.1 X maintenance. Therefore, concerning central effects on the pattern of LH secretion, the lactating sow and cyclic gilt models are apparently not comparable.

6.3 Local effects at the ovary

Other experimental models dealt with the provision of additional dietary energy above maintenance requirements (flush feeding). Flowers et al. (1989) reported that gilts submitted to flush feeding prior to estrus exhibited increased ovulation rates. Associated with this dietary-induced enhancement of follicular growth were increases in plasma FSH and insulin, concentrations and episodic secretion of LH. It was suggested that changes in plasma insulin concentrations may mediate the effects of flush feeding on gonadotropin secretion. In a further study of the endocrinology of nutritional flushing, the findings of Beltranena et al. (1991) were consistent with the results of Flowers et al. (1989) that in the pig, increased episodic

LH secretion and elevated plasma insulin and IGF-I concentrations, acting independently or synergistically, play an important role in mediating the restoration of ovulation rate observed in response to nutritional flushing. Further evidence for the role of insulin as a mediator of the increased ovulation rate due to nutritional flushing came from the study of Cox et al. (1987), in which increasing dietary energy and administering insulin during the follicular phase enhanced ovulation rate. It was also reported that the increases in ovulation rate were not necessarily accompanied by changes in gonadotropins. Moreover, Cosgrove et al. (1992) demonstrated that realimentation over five days exerted effects at the ovary, in the absence of elevations in plasma LH concentrations, increasing follicular growth and estradiol synthesis. These effects may, in part, have been mediated by insulin, IGF-I, and other growth factors that were not measured in their study (EGF, TGF- α, and TGF-β). The level of feed restriction used was 30% of ad libitum intake, but the predicted increase in LH secretion to refeeding was restrained by use of the gonadotropin suppressant allyl trenbolone.

Taken together, these models suggest that gilts are an effective experimental model for studying the mechanisms mediating nutrition-reproduction interactions, and at least some of these effects are exerted at the ovarian level.

The results in Experiment 1 showed that feed restriction during the second week of the cycle decreased embryo survival, without affecting ovulation rate. Thus, the pattern of feed intake during the recruitment of follicles into the preovulatory pool may have important consequences for embryo survival, irrespective of the number of follicles finally selected for ovulation. Moreover, the pattern of rise in plasma progesterone after ovulation was also affected by feed restriction during the second week of the cycle. These data suggest that at least the maturing follicle and possibly the oocyte may have been affected by the later period of feed restriction applied (d 8-15 of the cycle), which would then mediate deleterious effects on embryo survival. The role of progesterone as a possible mediator of nutritionally-induced effects on early embryonic development has been extensively reviewed (Ashworth, 1994; Foxcroft, 1997). Therefore, the slower rise in progesterone after ovulation may have been responsible for the lower embryonic survival reported in HR gilts restricted in the second week of the cycle. Using the experimental model developed by Zak et al. (1997a), Jindal et al. (1996) reported that lower embryonic survival in sows restricted during the last week of a 28-day lactation was highly correlated to progesterone concentrations in early pregnancy.

The results of Experiment 1 led us to further study the possible mechanisms mediating the deleterious effects of feed restriction during the second week of the estrous cycle on gilt subsequent fertility. Again, based on studies in the lactating sow, suggesting that nutrition and metabolic state might act at the level of the follicle to influence oocyte maturation (Zak et al. 1997a,b; Yang et al., 2000) we chose to address this question in the gilt model by allowing fertilization to occur *in vivo* and then, recovered the early fertilized oocytes for culture *in vitro*, to assess further embryonic development. A similar approach had been used successfully in sheep to demonstrate lasting, progesterone-dependent, effects of previous nutrition on embryonic development (McEvoy et al., 1995a,b) The results of Experiment 2 did not show effect of treatment on embryo developmental competence, however fertilization rate was lower in HR compared to RH gilts. Higher fertilization rate in the RH gilts, provided with a high plane of nutrition (2.8 X maintenance) during the late luteal phase of the cycle, would be consistent with the concept of higher quality oocytes in this group, although indirect effects of nutritional treatments on oviductal function and sperm maturation may also be a factor. As in Experiment 1, ovulation rate was not different in the RH and HR treatments; however, in this study no differences in plasma progesterone were observed. However, progesterone results in Experiment 3 showed that there was a difference in the rate of rise in progesterone after the LH surge, which was slower in HR compared to RH and HR+I gilts, even though no effect of treatment was observed for progesterone at ovulation, 12 hours after ovulation or 48 hours after onset of estrus. Therefore, if blood sampling was performed more frequently (e.g. every 6 hours) as it was done in Experiment 3, it may have been possible to observe differences in progesterone in Experiment 2. Thus in future experiments of this kind, a more frequent set of blood sample may be essential for adequately characterizing difference in progesterone status.

In Experiment 3, the endocrine data suggested that the level of feed restriction used (2.1 X maintenance) during the late luteal phase did not affect plasma concentrations of metabolic hormones (insulin, IGF-I, leptin, total T3 and free T3), gonadotropins (LH and FSH) or steroid hormones (estradiol and progesterone) in HR compared to RH gilts. The pattern of LH secretion was clearly controlled by plasma progesterone concentrations. However, the pattern of feed restriction had lasting effects on ovarian function in the peri-estrus period (low estradiol peak, low preovulatory surges of LH and FSH, and lower rise in progesterone after the LH surge). These deleterious effects were largely counteracted by insulin treatment. Furthermore, ovulation rate was increased by insulin treatment. Irrespective of these very interesting differences in the endocrine status of gilts in the peri-ovulatory period, which may mediate differences in embryonic survival, no effect of treatment was observed in the developmental competence of early fertilized oocytes.

The endocrine events in the peri-estrus period suggested that latent deleterious effects of feed restriction during the second week of the cycle are mediated through effects on ovarian function. The 'metabolic milieu' in which follicular growth occurs significantly influences follicle and oocyte maturation. According to Tsafriri (1988), cytoplasmic and nuclear maturation of the oocyte are closely regulated by its follicular enviroment, and in turn, this interaction affects the ability of the oocyte to produced viable blastocysts (Thibault, 1977). Thus, if the availability of metabolic fuels (e.g. energy, amino acids) is low, the oocyte may be exposed to a less mature follicular environment before ovulation, and subsequent cytoplasmic and nuclear maturation may be impeded. Nutritional effects acting through such mechanisms would be consistent with the lack of measurable differences in circulating hormones (insulin, IGF-I, leptin, total and free T3) that might be expected to mediate nutritional effects through classic endocrine mechanisms. Also the ability of the follicle to luteinize and synthesize progesterone, as demonstrated as an extension of Experiment 3 in our laboratory by J. Mao, may be compromised. Therefore, the experimental model described in the present thesis is appropriate and efficient to study the effects of nutrition on ovarian function.

An interesting finding in Experiment 1 was the placental-embryo association. Indeed, as one gilt had 26 corpora lutea and 26 embryos at day 28 of pregnancy, it was inevitable that uterine space after day 28 of pregnancy would be the major factor limiting litter size and fetal development. Hence, embryo-placental associations are being further investigated in our laboratory in the research project of S. Town.

Future studies using the cyclic gilt model established should be directed towards the investigation of intraovarian growth factors, such as IGF-I (as its synthesis in the ovary is independent of peripheral levels; Charlton et al., 1993), transforming growth factor β (TGFβ, especially inhibin and activin), and the IGF binding proteins. Moreover, assuming that the developmental competence of early fertilized oocytes may not be compromised, allowing embryos to develop until implantation (day 12 of pregnancy) *in vivo* may help to define the time at which embryo development is first affected by previous nutritional treatment.

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APPENDICES

APPENDIX 3.1 Body weight (BW) and backfat (BF) at 160 days of age and weekly (W) growth rate (GR) of gilts in Experiment 1.

Gilt #	BW160	BF 160	W1	GR1	W2	GR2	W3
171	80	8.5	86	6	90	4	94
161	80	9.5	86	6	89	3	98
162	86	10	94	8	100	6	102
306	74	9	75	1	80	5	89
301	93	9	96	3	98	2	109
303	76	7.5	75	-1	80	5	91
300	100	9	106	6	107	1	117
302	101	10	106	5	108	2	114
307	88	9	96	8	97	1	107
213	80	9	88	8	91	3	100
180	75	8	84	9	87	3	95
184	76	7	85	9	87	2	95
280	95	9	100	5	105	5	110
281	95	10.5	103	8	109	6	114
282	95	10.5	101	6	109	8	112
292	91	9	93	2	101	8	108
294	90	6.5	97	7	100	3	108
293	91	8	98	7	106	8	119
320	86	9.5	92	6	97	5	107
321	95	9.5	97	2	100	3	105
322	106	8	111	5	115	4	124
351	110	13.5	113	3	116	3	121
356	102	16	104	2	107	3	114
354	103	14	107	4	111	4	116
377	89	8	93	4	99	6	103
411	98	12	97	-1	104	7	109
416	99	15.5	105	6	110	5	112
33	111	11	116	5	122	6	128
491	102	9	104	2	108	4	113
494	95	6	101	6	112	11	119
31	109	10	116	7	121	5	127
92	98	12	103	5	111	8	118

Gilt #	BW160	BF 160	W1	GR1	W2	GR2	W3
93	94	12.5	99	5	105	6	113
51	99	12	102	3	108	6	114
51x	87	9	93	6	96	3	103
54	91	7	96	5	100	4	108
90	88	9	93	5	100	7	107
91	97	12	100	3	106	6	113
94	90	13.5	92	2	100	8	108
130	91	11.5	96	5	103	7	110
131	116	14	123	7	129	6	135
135	109	13	113	4	121	8	127
96	88	10.5	94	6	101	7	108
121	95	8	100	5	106	6	112
123	90	7	96	6	103	7	110
300	106	13.5	108	2	111	3	115
301	100	9.5	104	4	107	3	115
304	104	12.5	106	2	109	3	110
262	117	112	126	9	128	2	136
264	116	10.5	112	-4	114	2	117
265	113	11	113	0	118	5	120
421	95	10	102	7	107	5	109
422	91	10	99	8	104	5	102
423	89	10.5	91	2	97	6	101
453	102	10	106	4	112	6	113
465	85	11.5	90	5	96	6	101
466	102	11	106	4	112	6	114
320	93	10.5	99	6	102	3	108
321	100	11	103	3	109	6	115
323	106	11	108	2	110	2	114
80	124	14	125	1	130	5	135
82	97	11	99	2	106	7	107
85	87	8.5	93	6	100	7	105
460	90	9	94	4	101	7	105
461	114	11.5	119	5	124	5	126
462	92	8.5	96	4	103	7	105
71	99	12	102	3	108	6	111
72	104	13	102	-2	109	7	113
76	91	10	98	7	99	1	104

Gilt #	GR3	W4	GR4	W5	GR5	W6	GR6
171	4	97	3	103	6	106	3
161	9	99	1	111	12	112	1
162	2	109	7	116	7	118	2
306	9	93	4	99	6	Treat.	
301	11	110	1	117	7	Treat.	
303	11	95	4	100	5	Treat.	
300	10	122	5	127	5	134	7
302	6	119	5	123	4	130	7
307	10	110	3	115	5	122	7
213	9	103	3	109	6	116	7
180	8	96	1	108	12	111	3
184	8	96	1	105	9	108	3
280	5	117	7	119	2	123	4
281	5	119	5	121	2	125	4
282	3	119	7	122	3	126	4
292	7	111	3	115	4	117	2
294	8	112	4	114	2	119	5
293	13	121	2	127	6	128	1
320	10	111	4	113	2	118	5
321	5	109	4	112	3	114	2
322	9	128	4	132	4	134	2
351	5	123	2	128	5	130	2
356	7	115	1	118	3	128	10
354	5	120	4	122	2	126	4
377	4	107	4	110	3	116	6
411	5	116	7	122	6	125	3
416	2	118	6	123	5	125	2
33	6	131	3	135	4	141	6
491	5	121	8	127	6	131	4
494	7	121	2	126	5	131	5
31	6	130	3	134	4	139	5
92	7	122	4	Treat.			

Gilt #	GR3	W4	GR4	W5	GR5	W6	GR6
93	8	116	3	Treat.			
51	6	119	5	125	6	131	6
51x	7	108	5	114	6	124	10
54	8	110	2	117	7	120	3
90	7	110	3	115	5	119	4
91	7	116	3	122	6	129	7
94	8	112	4	116	4	123	7
130	7	114	4	110	-4	123	13
131	6	139	4	140	1	148	8
135	6	132	5	128	-4	139	11
96	7	113	5	117	4	120	3
121	6	117	5	120	3	126	6
123	7	114	4	119	5	127	8
300	4	124	9	125	1	130	5
301	8	119	4	123	4	129	6
304	1	117	7	118	1	123	5
262	8	136	0			145	
264	3	123	6	130	7	138	8
265	2	121	1	122	1	131	9
421	2	113	4	119	6	116	-3
422	-2	110	8	113	3	114	1
423	4	104	3	109	5	109	0
453	1	116	3	121	5	125	4
465	5	107	6	110	3	116	6
466	2	118	4	124	6	128	4
320	6	110	2	114	4	126	12
321	6	119	4	121	2	129	8
323	4	117	3	121	4	127	6
80	5	141	6	Treat.			
82	1	116	9	Treat.			
85	5	109	4	Treat.			
460	4	111	6	114	3	119	5
461	2	131	5	136	5	139	3
462	2	110	5	113	3	113	0
71	3	116	5	122	6	Treat.	
72	4	118	5	123	5	Treat.	
76	5	109	5	116	7	Treat.	

Gilt #	W7	GR7	W8	GR8	W9	GR9	W10
171	109	3	115	6	Treat.		Treat.
161	119	7	124	5	Treat.		Treat.
162	124	6	132	8	Treat.		Treat.
306					Treat.		Treat.
301					Treat.		Treat.
303					Treat.		Treat.
300	Treat.				Treat.		Treat.
302	Treat.				Treat.		Treat.
307	Treat.				Treat.		Treat.
213	119	3	126	7	Treat.		Treat.
180	113	2	123	10	Treat.		Treat.
184	113	5	119	6	Treat.		Treat.
280	128	5	Treat.		Treat.		Treat.
281	129	4	Treat.		Treat.		Treat.
282	130	4	Treat.		Treat.		Treat.
292	122	5	Treat.		Treat.		Treat.
294	125	6	Treat.		Treat.		Treat.
293	133	5	Treat.		Treat.		Treat.
320	115	-3	118	3	118	0	Treat.
321	118	4	120	2	123	3	Treat.
322	136	2	139	3	142	3	Treat.
351	135	5	Treat.		Treat.		
356	129	1	Treat.		Treat.		
354	126	0	Treat.		Treat.		
377	120	4	127	7	Treat.		
411	131	6	137	6	Treat.		
416	131	6	136	5	Treat.		
33	Treat.						
491	Treat.						
494	Treat.						
31	147	8	Treat.				
92							

Gilt #	W7	GR7	W8	GR8	W9	GR9	W10
93							
51	134	3	Treat.				
51x	127	3	Treat.				
54	126	6	Treat.				
90	124	5			133		
91	132	3			141		
94	127	4	136	9	136		
130	125	2	127	2	Treat		
131	152	4	162	10	Treat		
135	141	2	148	7	Treat		
96	126	6	130	4	133	Treat	
121	131	5	135	4	138	Treat	
123	131	4	134	3	137	Treat	
300	Treat.						
301	Treat.						
304	Treat.						
262	140	-5	150	10	Treat.		
264	140	2	149	9	Treat.		
265	134	3	139	5	Treat.		
421	Treat.						
422	Treat.						
423	Treat.						
453	Treat.						
465	Treat.						
466	Treat.						
320	126	0	129	3	135	6	141
321	131	2	140	9	144	4	148
323	131	4	132	1	132	0	147
80							
82							
85							
460	125	6	132	7	Treat.		
461	147	8	153	6	Treat.		
462	119	6	123	4	Treat.		
320	GR10	6	W11	Treat.			
321	GR10	4	W11	Treat.			
323	GR10	15	W11	Treat.			

Appendix 3.2 Age, body weight and backfat at pubertal estrus of gilts in Experiment 1.

Gilt #	BW	Age	BF	Gilt #	BW	Age	BF
171	95	179	9.5	304	110	172	12.5
161	111	192	11	262	124	171	10
162	101	177	10	264	130	193	10.5
306	80	162	805	265	124	193	10
301	98	163	9.5	421	105	175	11
303	80	163	8	422	104	175	11
300	105	154	9	423	91	161	10.5
302	103	155	10	453	112	172	10
307	103	167	9	465	101	182	11
213	100	180	8	466	106	166	11
180	106	195	9	320	125	208	11
184	99	191	8	321	119	181	11.5
280	107	167	9	323	127	205	12
281	119	180	10	80	125	165	14
282	114	171	11	82	99	167	11
292	107	180	905	85	93	167	8.5
294	97	164	6.5	460	114	194	10
293	121	187	9.5	461	136	194	11
320	112	186	10.5	462	113	194	9.5
321	114	193	11	71	102	165	12
322	124	176	8	72	101	164	13
351	122	180	17	76	98	171	10
356	115	184	15	51x	103	182	10
354	111	172	12	54	110	188	8.5
377	110	194	9	90	102	176	9
411	118	191	10	91	115	185	11.5
416	123	194	14	94	112	192	11
33	122	174	13	130	110	180	9.5
491	112	180	10	131	140	187	16
494	112	176	9.5	135	127	180	12
31	126	181	11	96	115	192	11
92	99	163	10	121	124	202	9.5
93	92	161	12	123	110	181	8
51	114	186	12	300	115	172	13
301	109	175	10.5				

Appendix 3.3 Adjustments of daily feed intake of gilts based on body weight (kg). within a 10 kg range.

Body weight (kg)	Feed intake (kg)
90	2.1
100	2.2
110	2.3
120	2.4
130	2.4
140	2.5

Appendix 4.1 Body weight (BW) and backfat (BF) at 160 days of age and weekly (W) growth rate (GR) of gilts in Experiment 2.

Gilt #	BW 160	BF 160	W1	GR1	W2	GR2	W3
290	90	7	95	5	99	4	104
294	110	9.5	118	8	121	3	126
400	99	8.5	104	5	108	4	111
401	93	7	96	3	100	4	104
403	89	8	92	3	97	5	102
404	100	7	102	2	106	4	110
430	86	7	92	6	97	5	104
432	103	9.5	106	3	110	4	115
483	120	9.5	121	1	127	6	133
485	103	8.5	109	6	116	7	115
11	107	9	110	3	118	8	120
15	97	11.5	100	3	101	1	106
60	100	9	98	-2	104	6	104
63	105	9	109	4	111	2	117
163	102	8	107	5	112	5	116
165	95	10	98	3	104	6	107
210	92	8.5	97	5	104	7	106
211	107	7	108	1	110	2	115
171 X	87	9	94	7	97	3	103
178	95	9.5	100	5	103	3	108
374	105	12	111	6	118	7	123
376	102	11	108	6	112	4	120
411	84	8	83	-1	87	4	96
412	94	9	97	3	105	8	113
404	88	7	96	8	100	4	103
407	92	8	90	-2	96	6	103
391	105	12	110	5	116	6	117
392	102	13	106	4	110	4	113
400	93	9.5	97	4	101	4	95
401	94	9	101	7	107	6	113
480	98	8.5	105	7	114	9	110
482	105	8	104	-1	114	10	116
372	94	9	99	5	104	5	108
375	106	12.5	109	3	112	3	119

Gilt #	BW 160	BF 160	W1	GR1	W2	GR2	W3
450	106	11	110	4	113	3	120
454	108	11	115	7	123	8	123
480	101	7.5	103	2	116	13	120
482	105	9	109	4	114	5	116
Gilt #	**GR3**	**W4**	**GR4**	**W5**	**GR5**	**W6**	**GR6**
290	5	108	4	118	10	111	-7
294	5	129	3	132	3	138	6
400	3	114	3	118	4	121	3
401	4	110	6	113	3	115	2
403	5	104	2	109	5	112	3
404	4	114	4	119	5	122	3
430	7	107	3	111	4	113	2
432	5	118	3	121	3	125	4
483	6	134	1	135	1	137	2
485	-1	122	7	126	4	129	3
11	2	127	7	129	2	131	2
15	5	114	8	116	2	119	3
60	0	112	8	114	2	115	1
63	6	121	4	123	2	126	3
163	4	121	5	129	8	132	3
165	3	110	3	117	7	124	7
210	2	109	3	114	5	119	5
211	5	119	4	125	6	127	2
171 X	6	109	6	113	4	120	7
178	5	111	3	111	0	119	8
374	5	122	-1	124	2	124	0
376	8	124	4	128	4	131	3
411	9	100	4	105	5	108	3
412	8	118	5	121	3	126	5
404	3	112	9	114	2	123	9
407	7	110	7	115	5	119	4
391	1	127	10	134	7	141	7
392	3	118	5	126	8	133	7
400	-6	103	8	105	2	111	6
401	6	118	5	120	2	122	2
480	-4	113	3	118	5	Treat.	

Gilt #	GR3	W4	GR4	W5	GR5	W6	GR6
482	2	122	6	128	6	Treat.	
372	4	111	3	122	11	Treat.	
375	7	114	-5	123	9	Treat.	
450	7	124	4	127	3	Treat.	
454	0	126	3	130	4	Treat.	
480	4	126	6	130	4	Treat.	
482	2	121	5	127	6	Treat.	
Gilt #	W7	GR7	W8	GR8	W9	GR9	W10
290	118	7	Treat.		Treat.		
294	141	3	Treat.		Treat.		
400	133	12	Treat.		Treat.		
401	120	5	Treat.		Treat.		
403	118	6	121	3	124	3	129
404	126	4	130	4	132	2	139
430	116	3	121	5	121	0	129
432	129	4	131	2	135	4	141
483	140	3	145	5	156	11	Treat.
485	134	5	138	4	148	10	Treat.
11	134	3	136	2	147	11	Treat.
15	125	6	129	4	136	7	Treat.
60	120	5	123	3	128	5	Treat.
63	130	4	133	3	140	7	Treat.
163	135	3	137	2	Treat.		
165	126	2	129	3	Treat.		
210	121	2	127	6	Treat.		
211	138	11	136	-2	Treat.		
171 X	127	7	129	2	Treat.		
178	124	5	129	5	Treat.		
374	129	5	Treat.		Treat.		
376	136	5	Treat.		Treat.		
411	Treat.				Treat.		
412	Treat.				Treat.		
404	Treat.				Treat.		
407	Treat.				Treat.		
391	Treat.				Treat.		
392	Treat.				Treat.		
400	Treat.				Treat.		

Gilt #	W7	GR7	W8	GR8	W9	GR9	W10
401	Treat.				Treat.		
480					Treat.		
482					Treat.		
372					Treat.		
375					Treat.		
450					Treat.		
454					Treat.		
480					Treat.		
482					Treat.		
Gilt #	GR10	W11					
403	5	Treat.					
404	7	Treat.					
430	8	Treat.					
432	6	Treat.					

Appendix 4.2 Age, body weight and backfat at pubertal estrus of gilts in Experiment 2.

Gilt #	Age	BW	BF	Gilt #	Age	BW	BF
472	180	95	8	15	192	112	12.5
474	174	93	-	163	184	116	9
401	194	110	11.5	165	184	105	10
406	198	112	10	210	193	114	10
81	180	95	9	211	193	-	8
86	165	105	9	171X	195	113	9
84	165	102	8	178	195	111	10
85	176	115	10	400	169	99	9.5
442	181	106	8	401	163	95	9
444	185	106	-	411	178	88	8
463	188	112	-	412	164	95	9
464	195	107	8	163	184	116	9
90	172	121	14	165	184	105	10
92	163	111	11	210	193	114	10
93	160	104	12	211	193	-	8
94	163	106	11	171X	195	113	9
70	165	104	11	178	195	111	10
72	186	120	9.5	400	169	99	9.5
320	165	95	8.5	401	163	95	9
321	164	107	10	411	178	88	8
290	184	110	8	412	164	95	9
294	171	121	9.5	483	199	137	10.5
400	183	111	8.5	485	182	115	9.5
401	183	104	7	60	196	114	8
480	164	100	7.5	63	197	126	9.5
482	163	105	9	11	190	124	10
403	201	112	9.5	15	192	112	12.5
404	203	122	9	432	204	124	10.5
430	194	110	8	-	-	-	-

Appendix 5.1 Body weight (BW) and backfat (BF) at 160 days of age and weekly (W) growth rate (GR) of gilts in Experiment 3.

Gilt #	BW160	BF 160	W1	GR1	W2	GR2	W3
112	109	10.5	108	-1	113	5	117
116	89	9	93	4	93	0	100
119	99	8.5	103	4	109	6	114
131	120	10	125	5	124	-1	131
132	117	11	120	3	123	3	127
133	118	11	121	3	123	2	126
240	87	8	88	1	92	4	100
246	103	7	100	-3	104	4	109
247	103	9.5	102	-1	106	4	112
250	98	11	101	3	104	3	109
251	99	8	100	1	105	5	109
254	98	8.5	100	2	103	3	106
320	103	11	102	-1	110	8	114
321	93	12	90	-3	99	9	98
323	103	12.5	104	1	109	5	113
460	113	12.5	114	1	116	2	126
461	107	8.5	111	4	115	4	119
464	105	11	109	4	110	1	117
470	80	8	87	7	92	5	96
471	84		89	5	96	7	98
474	78	7.5	79	1	82	3	90
446	92	10	97	5	101	4	106
453	78	7.5	84	6	90	6	98
456	88	12	91	3	95	4	102
443	103	12.5	110	7	113	3	120
445	98	10.5	102	4	104	2	112
447	94	8.5	96	2	100	4	105
462	110	10	113	3	118	5	121
463	95	9	99	4	104	5	108
469	84	8	88	4	89	1	100
465	98	10	102	4	106	4	114
472	100	9	108	8	113	5	117
473	93	9	93	0	101	8	106
131	109	13	115	6	119	4	124

Gilt #	BW160	BF 160	W1	GR1	W2	GR2	W3
133	95	9.5	97	2	107	10	112
134	87	10	95	8	100	5	104
120	90	9.5	96	6	103	7	106
121	104	8	107	3	111	4	115
122	93	10	101	8	109	8	113
213	83	9	91	8	98	7	101
214	93	12	98	5	102	4	108
216	100	11	106	6	109	3	116
481	92	10	96	4	100	4	109
482	81	9	81	0	78	-3	90
483	99	13	105	6	107	2	113
451	77	9	84	7	87	3	95
454	78	9	84	6	87	3	90
457	79	9	85	6	87	2	90
230	104	13	110	6	116	6	119
231	106	12	112	6	118	6	124
232	94	9	101	7	110	9	114
210	106	12	111	5	117	6	121
130	97	11	103	6	110	7	117
135	78	11	85	7	92	7	100
123	102	9	110	8	115	5	115
124	94	10	103	9	108	5	112

Gilt #	GR3	W4	GR4	W5	GR5	W6	GR6
112	4	123	6	128	5	131	3
116	7	103	3	107	4	111	4
119	5	118	4	124	6	129	5
131	7	132	1	133	1	142	9
132	4	129	2	134	5	138	4
133	3	133	7	135	2	137	2
240	8	101	1	110	9	111	1
246	5	114	5	120	6	124	4
247	6	115	3	123	8	126	3
250	5	116	7	122	6	129	7
251	4	117	8	124	7	126	2
254	3	111	5	122	11	128	6
320	4	120	6	125	5	129	4
321	-1	96	-2	103	7	108	5
323	4	116	3	121	5	126	5
460	10	122	-4	126	4	Treat.	
461	4	121	2	127	6	Treat.	

Gilt #	GR3	W4	GR4	W5	GR5	W6	GR6
133	5	118	6	120	2	124	4
134	4	109	5	112	3	117	5
120	3	115	9	120	5	122	2
121	4	120	5	123	3	122	-1
122	4	115	2	117	2	124	7
213	3	108	7	109	1	110	1
214	6	119	11	118	-1	123	5
216	7	119	3	124	5	127	3
481	9	113	4	116	3	121	5
482	12	97	7	100	3	105	5
483	6	117	4	120	3	125	5
451	8	98	3	103	5	107	4
454	3	95	5	101	6	105	4
457	3	94	4	99	5	100	1
230	3	128	9	128	0	132	4
231	6	129	5	134	5	137	3
232	4	118	4	123	5	126	3
210	4	127	6	130	3	134	4
130	7	125	8	127	2	132	5
135	8	105	5	107	2	111	4
123	0	111	-4	118	7	123	5
124	4	116	4	122	6	122	0
464	7	118	1	121	3	Treat.	
470	4	102	6	106	4	110	4
471	2	121	23	124	3	128	4
474	8	94	4	98	4	100	2
446	5	108	2	112	4	116	4
453	8	102	4	107	5	109	2
456	7	105	3	110	5	112	2
443	7	121	1	127	6	131	4
445	8	114	2	118	4	123	5
447	5	106	1	111	5	115	4
462	3	122	1	112	-10	117	5
463	4	111	3	117	6	125	8
469	11	105	5	109	4	114	5
465	8	116	2	119	3	123	4
472	4	120	3	126	6	128	2
473	5	110	4	115	5	119	4
131	5	129	5	129	0	132	3

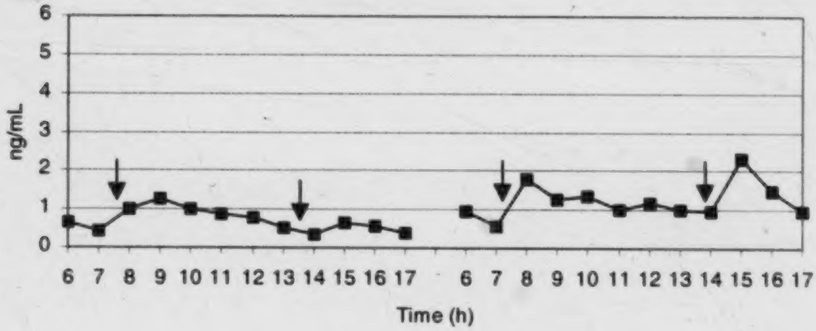
Gilt #	W7	GR7	W8	GR8	W9	GR9	W10
112	137	6	138	1	141	3	144
116	119	8	121	2	118	-3	123
119	138	9	144	6	143	-1	146
131	149	7	150	1	154	4	156
132	147	9	148	1	150	2	152
133	146	9	150	4	148	-2	151
240	112	1	116	4	Treat.		
246	123	-1	132	9	Treat.		
247	125	-1	130	5	Treat.		
250	128	-1	128	0	Treat.		
251	130	4	131	1	Treat.		
254	127	-1	129	2	Treat.		
320	132	3	134	2	Treat.		
321	112	4	114	2	Treat.		
323	129	3	127	-2	Treat.		
460							
461							
464							
470	116	6	119	3	Treat.		
471	131	3	133	2	Treat.		
474	108	8	112	4	Treat.		
446	121	5	124	3	Treat.		
453	117	8	120	3	Treat.		
456	117	5	121	4	Treat.		
443	133	2	137	4	Treat.		
445	123	0	128	5	Treat.		
447	118	3	123	5	Treat.		
462	123	6	127	4	Treat.		
463	125	0	128	3	Treat.		
469	117	3	121	4	Treat.		
465	124	1	128	4	135	7	Treat.
472	131	3	136	5	139	3	Treat.
473	122	3	128	6	134	6	Treat.
131	139	7	144	5	Treat.		
133	130	6	136	6	Treat.		
134	122	5	128	6	Treat.		
120	127	5	132	5	Treat.		

Gilt #	W7	GR7	W8	GR8	W9	GR9	W10
121	126	4	132	6	Treat.		
122	130	6	136	6	Treat.		
213	118	8	126	8	Treat.		
214	130	7	135	5	Treat.		
216	135	8	140	5	Treat.		
481	121	0	126	5	131	5	134
482	109	4	115	6	120	5	122
483	128	3	131	3	132	1	140
451	109	2	115	6	121	6	124
454	110	5	115	5	121	6	129
457	105	5	110	5	115	5	120
230	139	7	145	6	145	0	Treat.
231	141	4	145	4	148	3	Treat.
232	131	5	137	6	140	3	Treat.
210	139	5	146	7	146	0	Treat.
130	138	6	144	6	148	4	Treat.
135	118	7	123	5	126	3	Treat.
123	129	6	135	6	138	3	Treat.
124	126	4	133	7	137	4	Treat.
Gilt #	GR10	W11	GR11	W12	GR12	W13	
481	3	139	5	144	5	150	
482	2	127	5	132	5	137	
483	8	141	1	146	5	154	
451	3	126	2	132	6	146	
454	8	127	-2	135	8	138	
457	5	122	2	125	3	132	
112	3	Treat.					
116	5	Treat.					
119	3	Treat.					
131	2	Treat.					
132	2	Treat.					
133	3	Treat.					

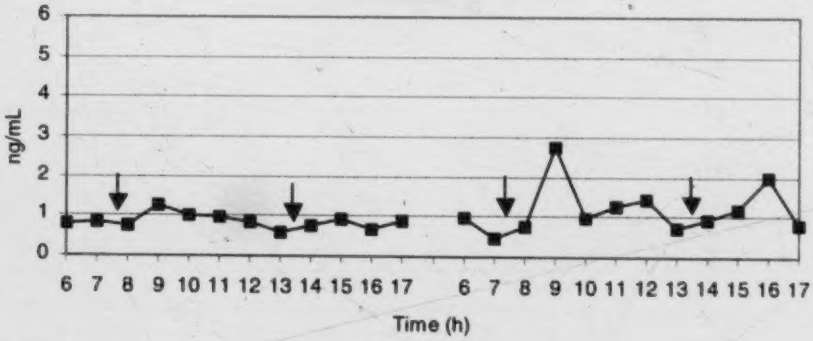
Appendix 5.2 Age, body weight and backfat at pubertal estrus of gilts in Experiment 3.

Gilt #	Age	BW	BF	Gilt #	Age	BW	BF
240	177	102	8	122	169	101	10
246	173	109	7	213	181	101	9
247	174	112	9.5	214	195	118	12
112	198	128	11	216	206	128	11
116	194	105	10	131	201	132	13
119	205	129	10	134	188	109	9.5
131	194	133	11	133	198	121	10
132	183	128	12	230	215	140	13
133	195	135	12	231	182	124	12
250	182	116	11	232	182	114	9
251	189	120	9	481	263	153	10
254	173	106	9	482	258	138	9
320	185	114	9.5	483	222	132	13
321	189	97	11	451	263	139	9
323	177	107	9.5	454	247	137	9
460	175	115	12.5	457	254	131	9
461	166	109	8.5	135	218	117	11
464	173	109	11		212	30.9	-
470	-	102	8	471	-	126	11.5
471	172	106	-	130	227	146	11
474	166	80	7.5	123	226	135	9
446	189	108	10	124	226	133	10
453	195	107	7.5	210	227	146	12
456	187	103	12	463	177	106	9
443	178	121	12.5	469	189	103	8
445	187	112	10.5	465	206	122	10
447	166	96	8.5	472	206	130	9
462	176	118	10	473	209	122	9
121	189	120	8	120	191	115	9.5

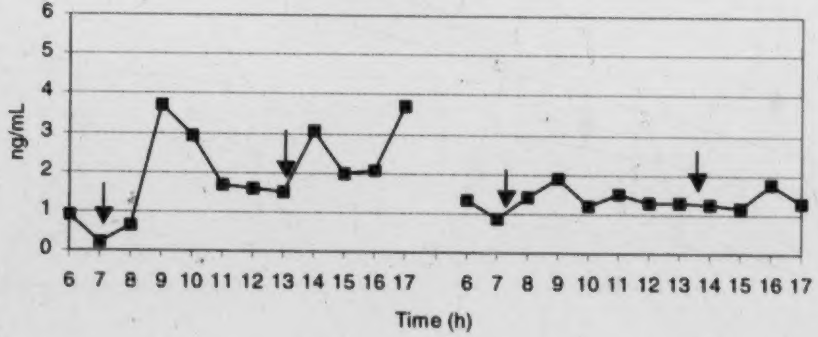
Appendix 5.3 Insulin profiles of three littermate gilts (nos. 451, 454 and 457) at d 15 and 16 of the cycle (arrows indicate time of feeding) and in the peri-estrus period in Experiment 3.



Pig # 451 - RH

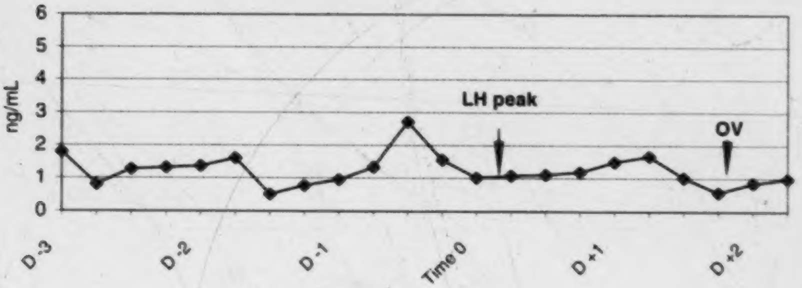


Pig # 454 - HR

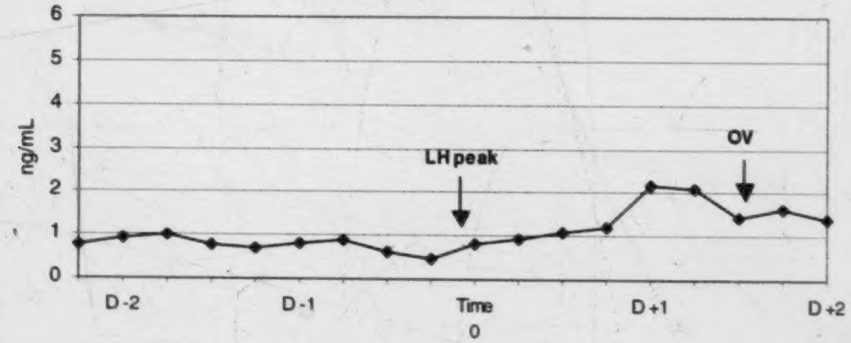


Pig # 457 - HR + I

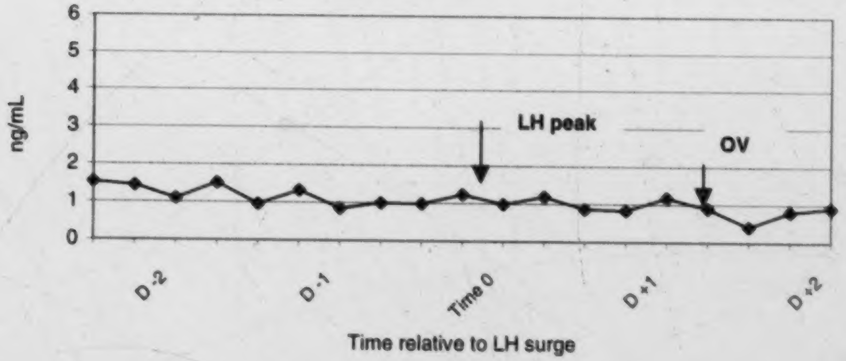
Pig # 451 - RH



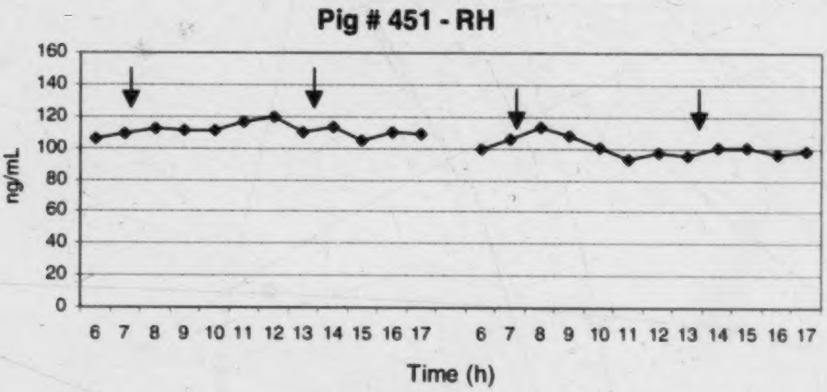
Pig # 454 - HR

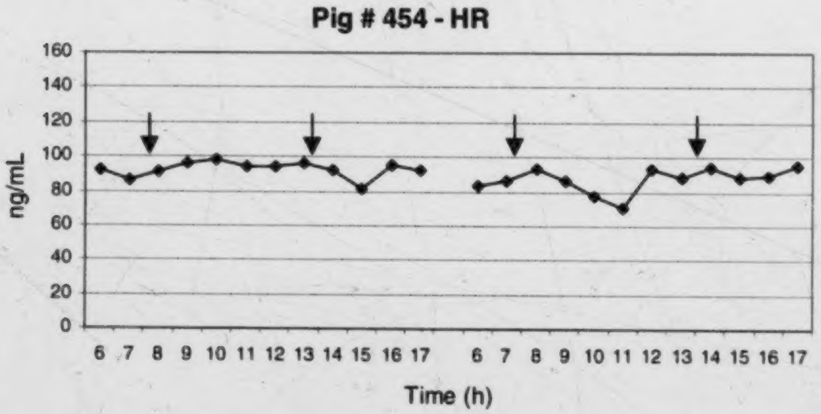


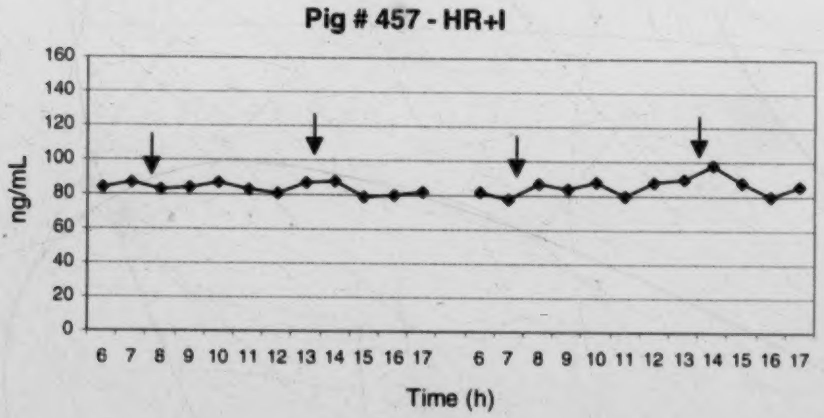
Pig # 457 HR+I

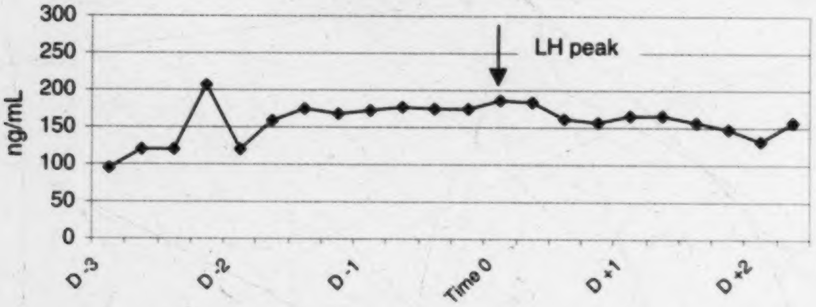


Appendix 5.4 IGF-I profiles of three littermate gilts (nos. 451, 454 and 457) at d 15 and 16 of the estrous cycle (arrows indicate time of feeding), and in the peri-estrus period in Experiment 3.

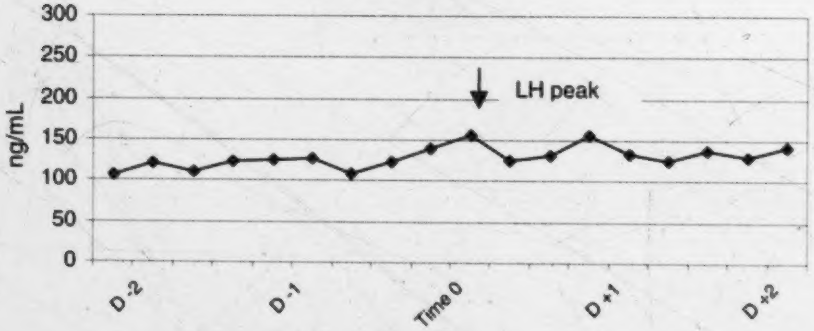

Pig # 451 - RH
ng/mL
0
20
40
60
80
100
120
140
160
6 7 8 9 10 11 12 13 14 15 16 17 6 7 8 9 10 11 12 13 14 15 16 17
Time (h)


Pig # 454 - HR
ng/mL
0
20
40
60
80
100
120
140
160
6 7 8 9 10 11 12 13 14 15 16 17 6 7 8 9 10 11 12 13 14 15 16 17
Time (h)

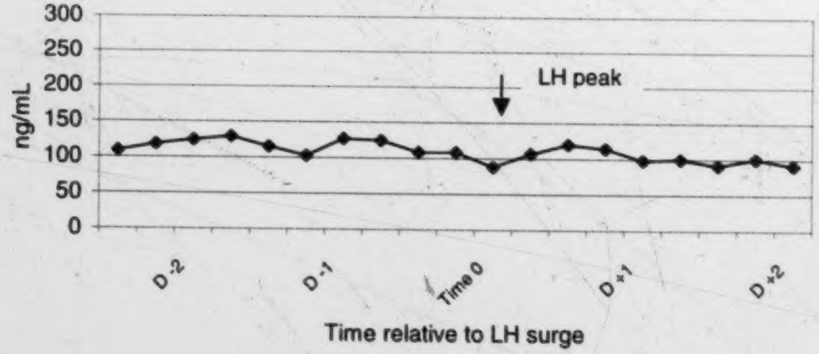

Pig # 457 - HR+I
ng/mL
0
20
40
60
80
100
120
140
160
6 7 8 9 10 11 12 13 14 15 16 17 6 7 8 9 10 11 12 13 14 15 16 17
Time (h)



Pig # 451 - RH

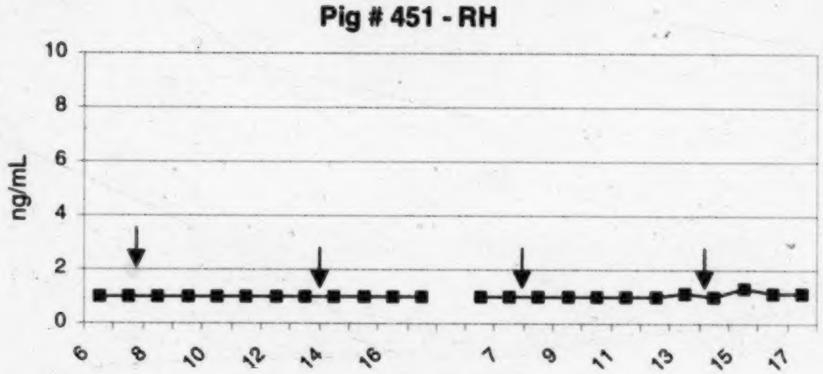


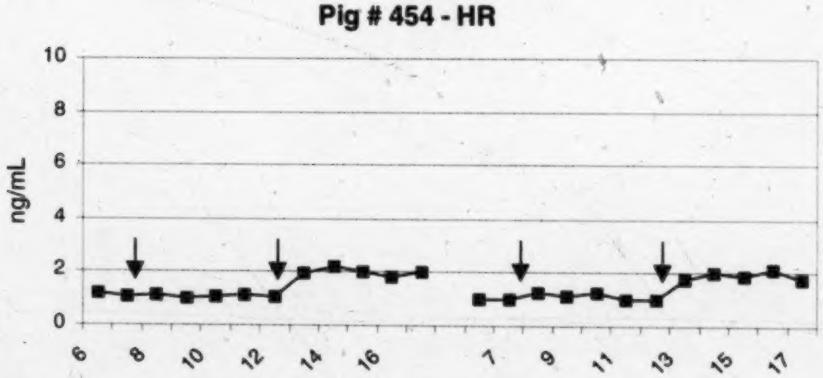
Pig # 454 - HR

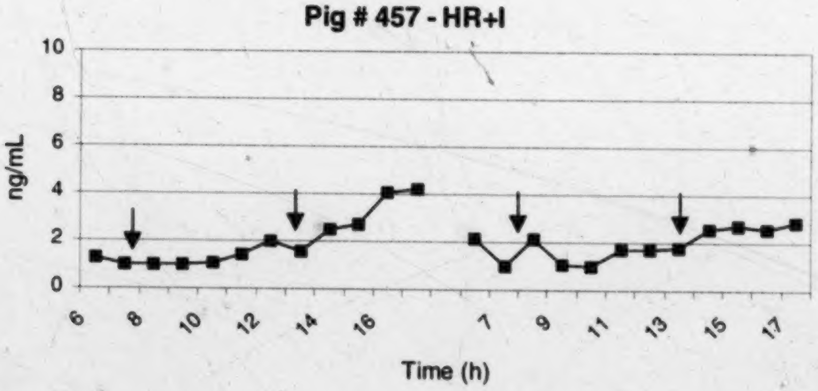


Pig # 457 - HR+I

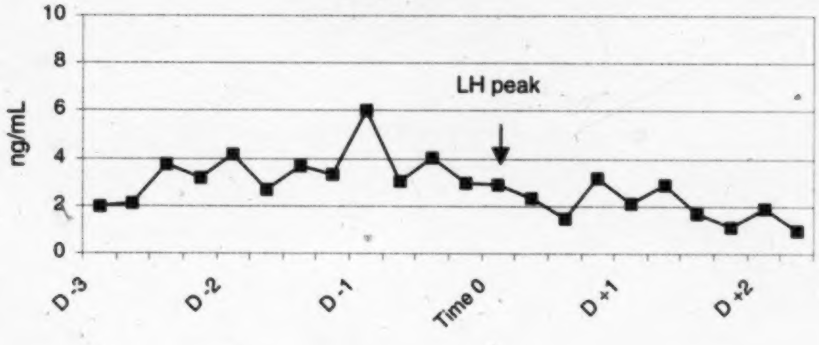
Appendix 5.5 Leptin profiles of three littermate gilts (nos. 451, 454 and 457) at d 15 and 16 of the estrous cycle (arrows indicate time of feeding), and in the peri-estrus period in Experiment 3.


Pig # 451 - RH

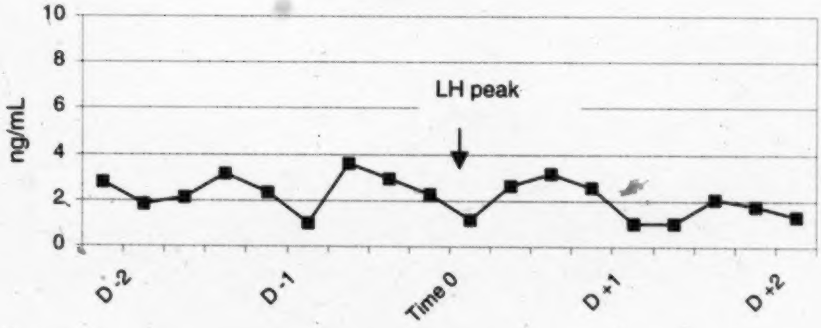

Pig # 454 - HR


Pig # 457 - HR+I

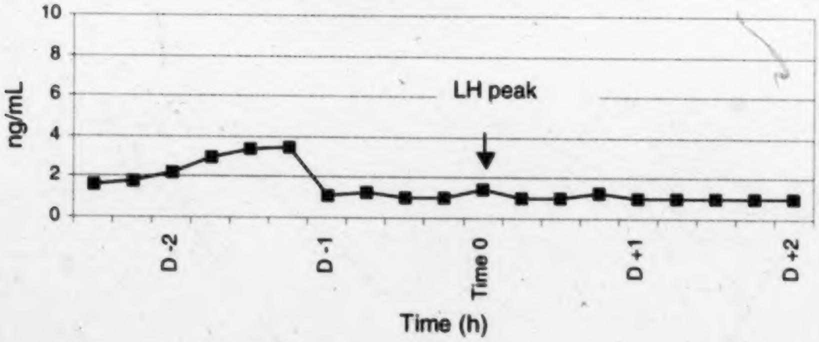
Pig # 451 - RH



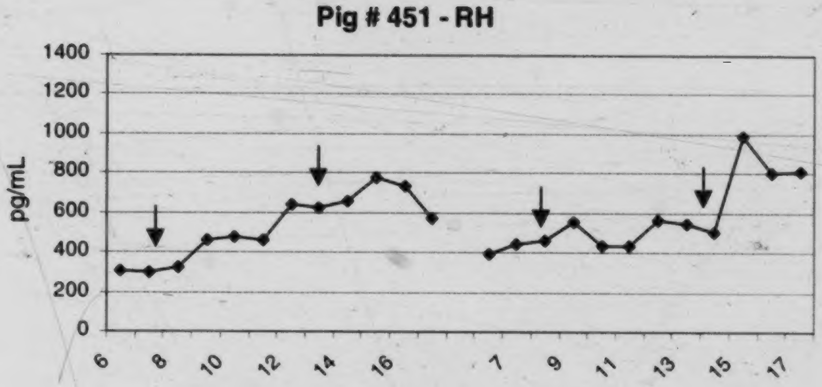
Pig # 454 - HR

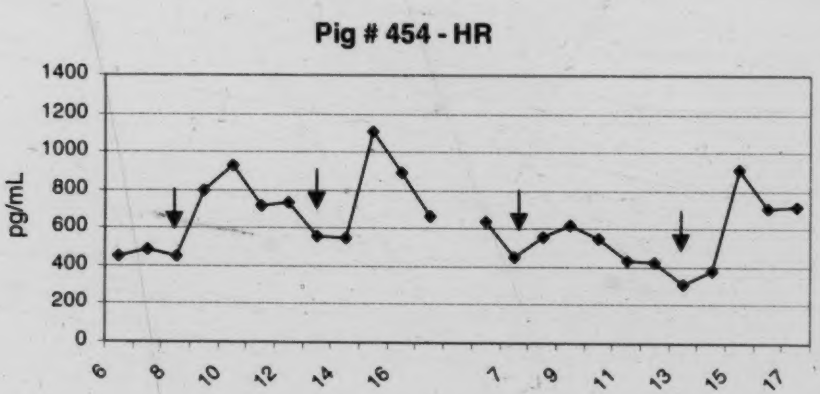


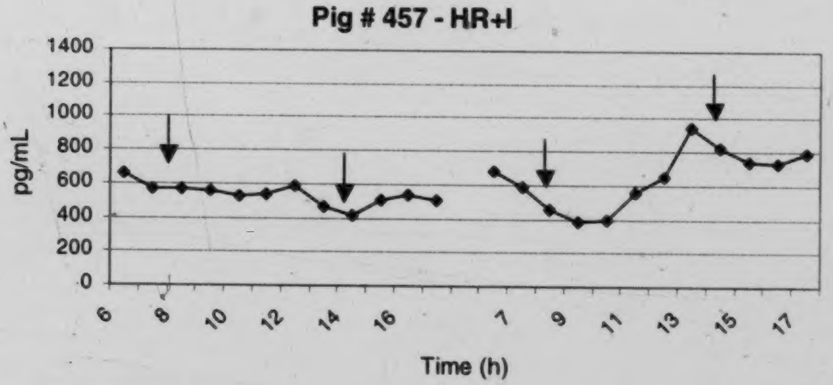
Pig # 457 - HR+I



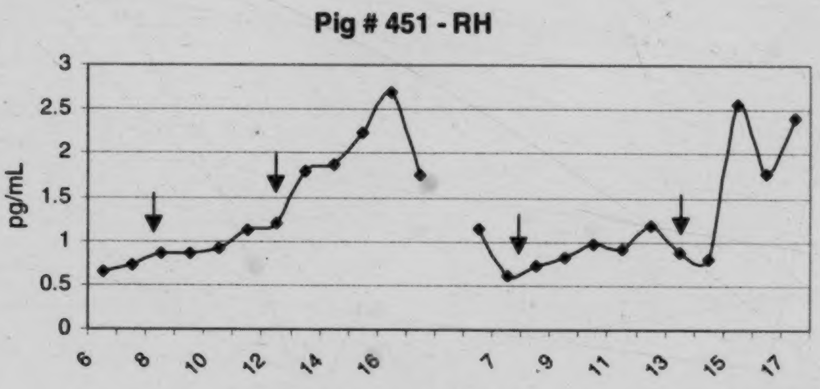
Appendix 5.6 Total T3 profiles of three littermate gilts (nos. 451, 454 and 457) at d 15 and 16 of the estrous cycle (arrows indicate time of feeding) in Experiment 3.

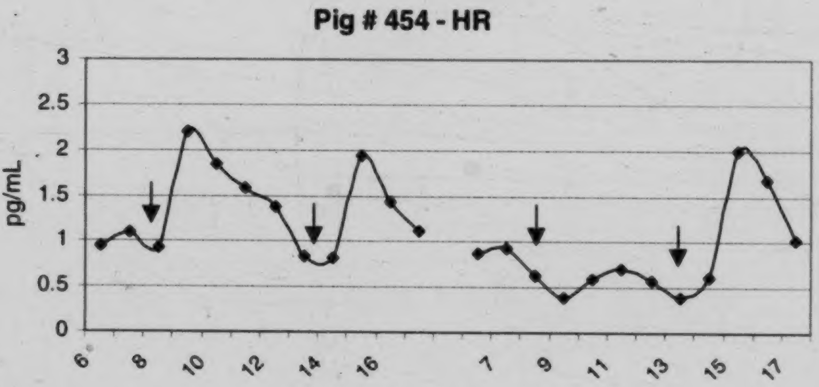

Pig # 451 - RH
pg/mL
1400
1200
1000
800
600
400
200
0
6
8
10
12
14
16
7
9
11
13
15
17

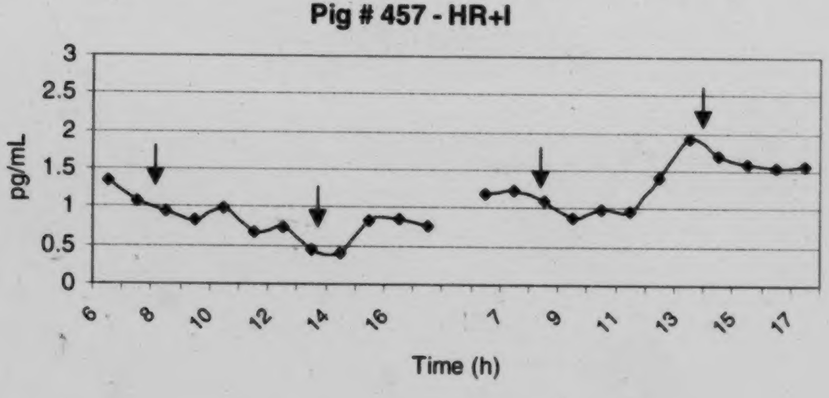

Pig # 454 - HR
pg/mL
1400
1200
1000
800
600
400
200
0
6
8
10
12
14
16
7
9
11
13
15
17


Pig # 457 - HR+I
pg/mL
1400
1200
1000
800
600
400
200
0
6
8
10
12
14
16
7
9
11
13
15
17
Time (h)

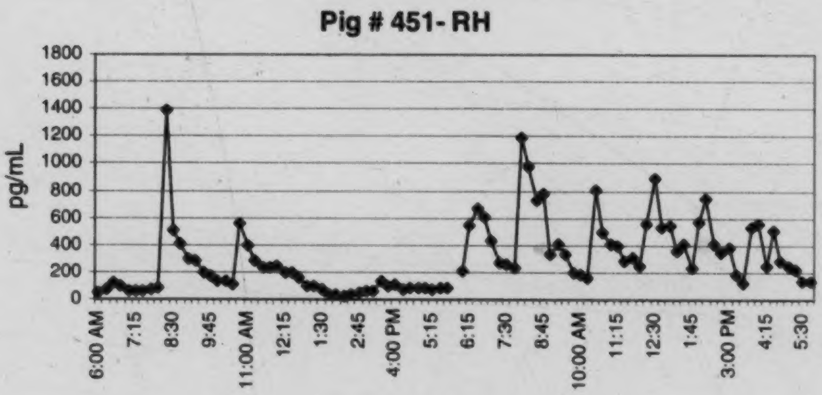
Appendix 5.7 Free T3 of three littermate gilts (nos. 451, 454 and 457) at d 15 and 16 of the estrous cycle (arrows represent time of feeding) in Experiment 3.

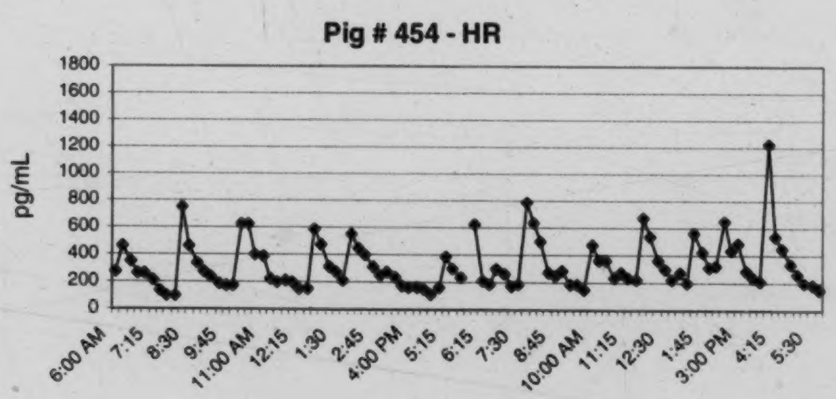

Pig # 451 - RH

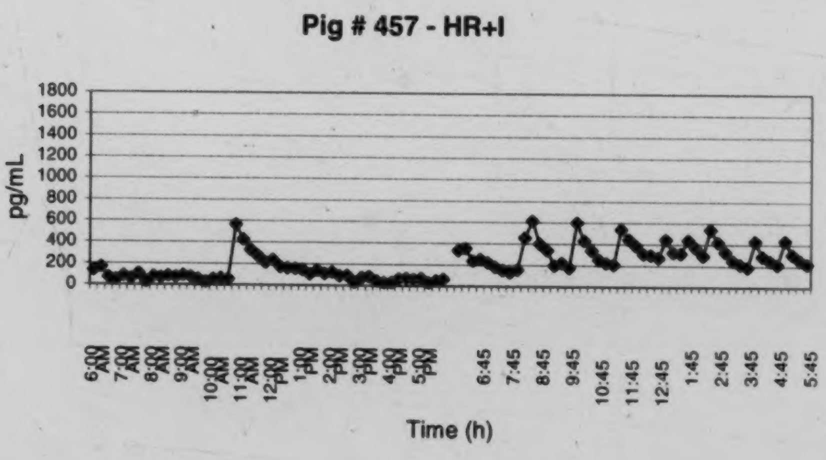

Pig # 454 - HR


Pig # 457 - HR+I

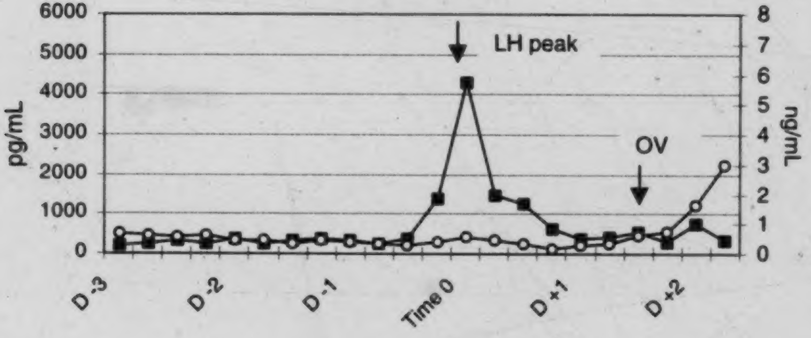
Appendix 5.8 LH profiles of three littermate gilts (451, 454 and 457) at d 15 and 16 of the estrous cycle in Experiment 3.


Pig # 451- RH
pg/mL
1800
1600
1400
1200
1000
800
600
400
200
0
6:00 AM
7:15
8:30
9:45
11:00 AM
12:15
1:30
2:45
4:00 PM
5:15
6:15
7:30
8:45
10:00 AM
11:15
12:30
1:45
3:00 PM
4:15
5:30

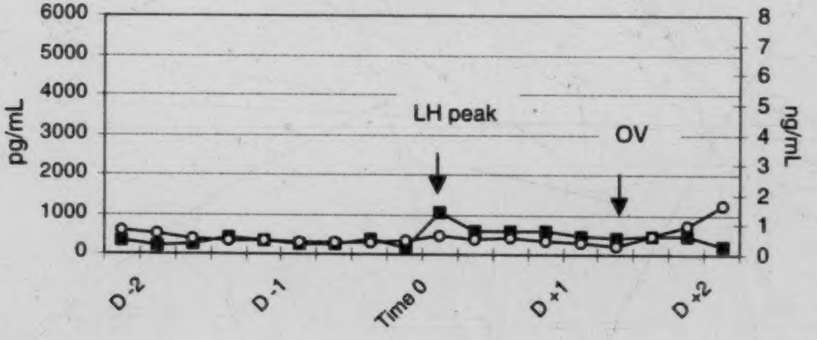

Pig # 454 - HR
pg/mL
1800
1600
1400
1200
1000
800
600
400
200
0
6:00 AM
7:15
8:30
9:45
11:00 AM
12:15
1:30
2:45
4:00 PM
5:15
6:15
7:30
8:45
10:00 AM
11:15
12:30
1:45
3:00 PM
4:15
5:30


Pig # 457 - HR+I
pg/mL
1800
1600
1400
1200
1000
800
600
400
200
0
6:00 AM
7:00 AM
8:00 AM
9:00 AM
10:00 AM
11:00 AM
12:00 PM
1:00 PM
2:00 PM
3:00 PM
4:00 PM
5:00 PM
6:45
7:45
8:45
9:45
10:45
11:45
12:45
1:45
2:45
3:45
4:45
5:45
Time (h)

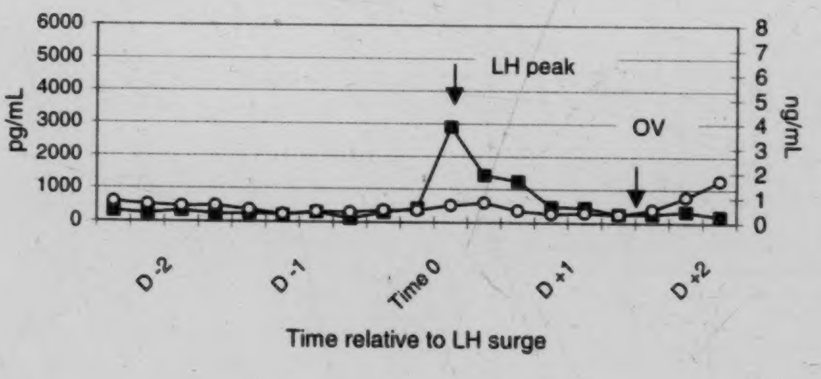
Appendix 5.9 LH and progesterone profiles of three littermate gilts (nos. 451, 454 and 457) in the peri-estrus period in Experiment 3.



Pig # 451 - RH

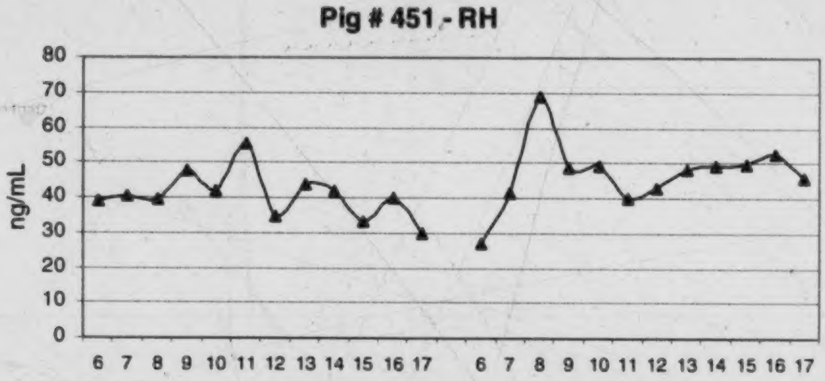


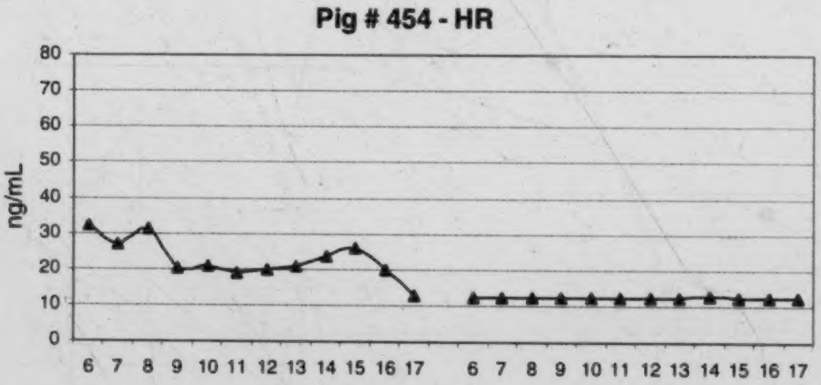
Pig # 454 - HR

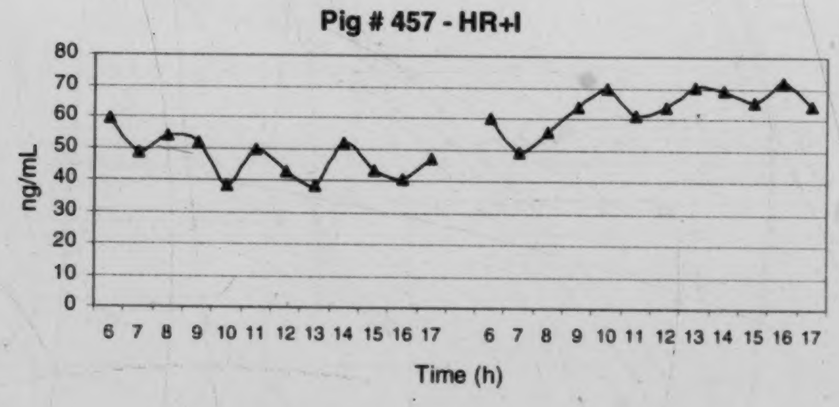


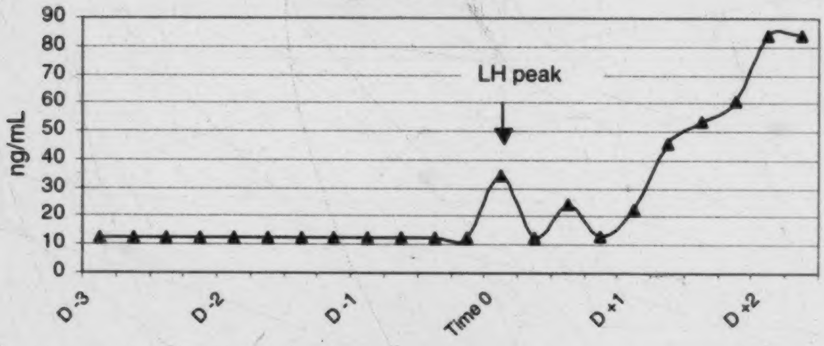
Pig # 457 - HR+I

Appendix 5.10 FSH profiles of three littermate gilts (nos. 451, 454 and 457) at d 15 and 16 of the estrous cycle and in the peri-estrus period in Experiment 3.

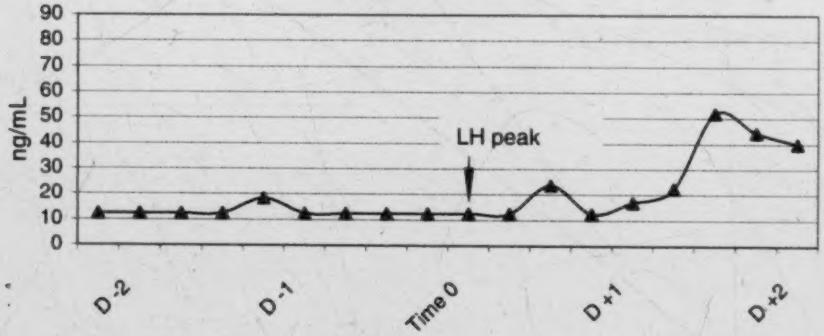

Pig # 451 - RH


Pig # 454 - HR

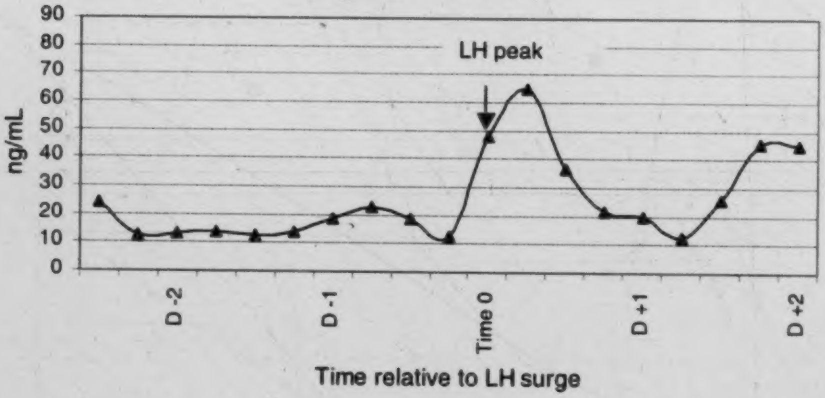

Pig # 457 - HR+I



Pig # 451 - RH



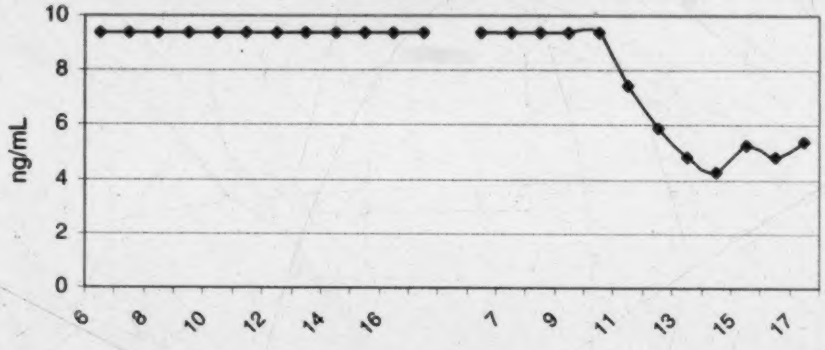
Pig # 454 - HR



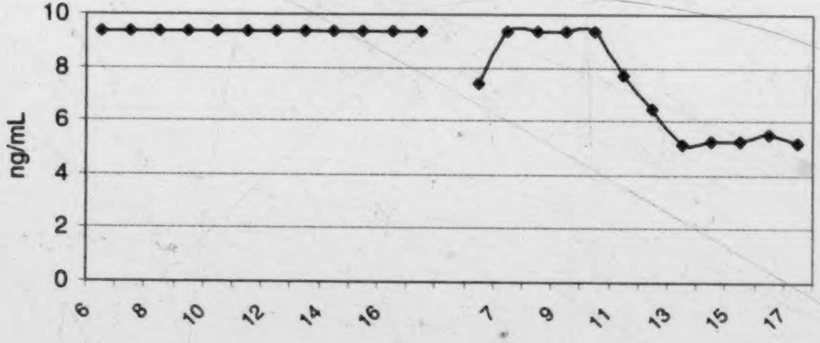
Pig # 457 - HR+I

Appendix 5.11 Progesterone profiles of three littermate gilts (nos. 451, 454 and 457) at d 15 and 16 of the estrous cycle in Experiment 3.

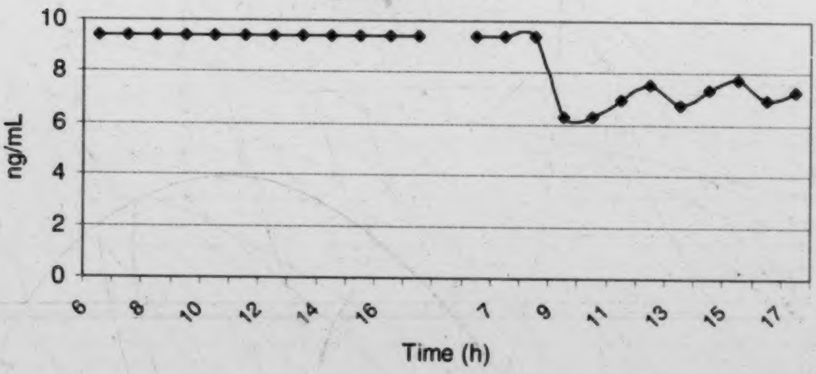
Pig # 451 - RH



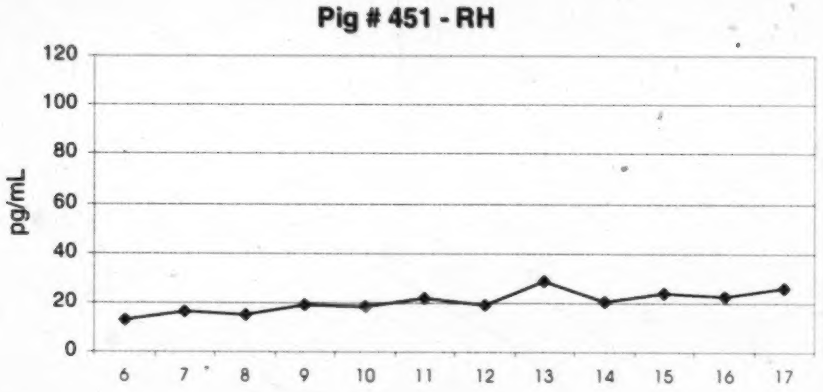
Pig # 454 - HR

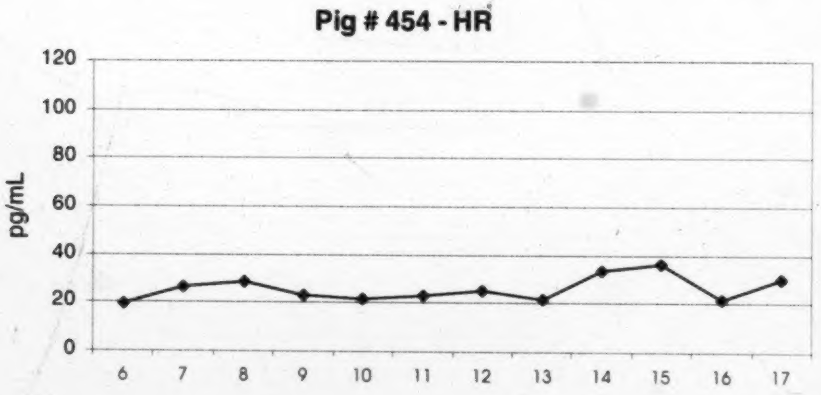


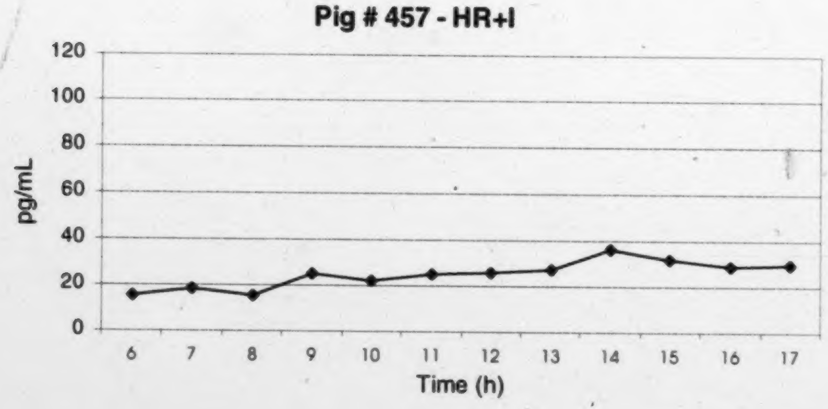
Pig # 457 - HR+I

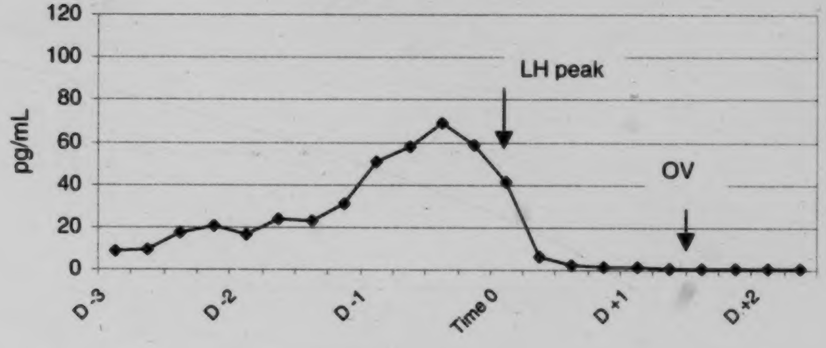


Appendix 5.12 Estradiol profiles of three littermate gilts (nos. 451, 454 and 457) at d 16 of the estrous cycle and in the peri-estrus period in Experiment 3.

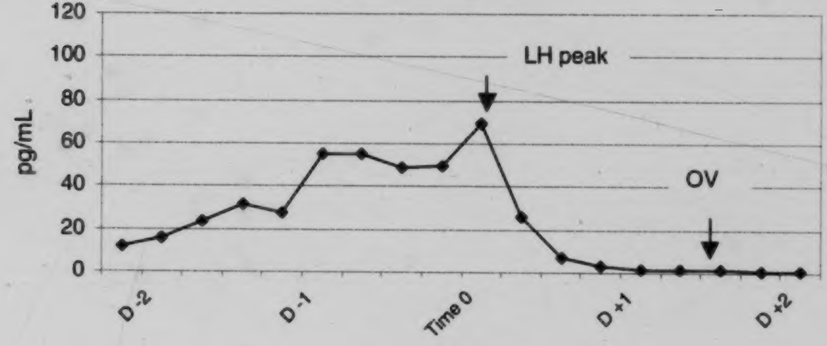

Pig # 451 - RH


Pig # 454 - HR

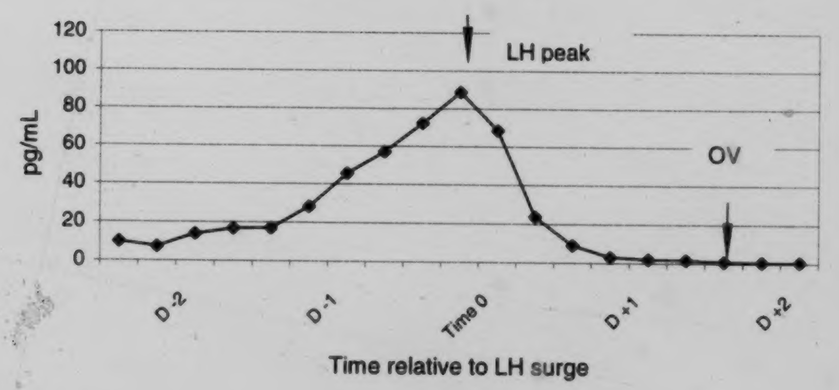

Pig # 457 - HR+I



Pig # 451 - RH



Pig # 454 - HR



Pig # 457 - HR+I